The Banting Memorial Lecture 1961

Concerning the Mechanisms of Insulin Action

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It is an inestimable privilege to be able to share with you some thoughts concerning the mechanisms of action of that hormone, which Banting and Best brought to light forty years ago this summer. I met Dr. Banting once, fleetingly, while I was a medical student at McGill. The talk that evening did not touch upon insulin but revolved around the social woes of the Europe of the early Thirties, and the impression I carried away was that of a man deeply concerned with the ethical aspects of human life and with the vast social forces operative in the world.

Soon thereafter it was my good fortune to do my metabolic apprenticeship under Samuel Soskin who was a student in Toronto with Charles Best and J. J. R. MacLeod. Because of this scientific kinship, I take particular pride in being allowed to speak in honor of Banting's name.

The original work to be discussed was done throughout with the most able collaboration of Dr. Maurice Goldstein, who now directs the laboratory which he joined in 1948. We had the help of a devoted group of graduate students among whom were Irving Fritz, Stanley Lang, Ira Wool and Sander Klein.

The chemical structure of insulin has been revealed by Sanger,[1] though we still lack an exact description of its spatial, tridimensional form. No definitive suggestion has come from this knowledge towards the solution of the riddle of its hormonal action. A recent hint worth pursuing concerns the possible reactions between the exterior disulfide linkages and the postulated cell receptor material. This portion may be, so to speak, the "business end" of the molecule.[2,3]

In broad terms, insulin acts catalytically. Vanishingly small amounts of hormone permit the increased utilization of vast amounts of glucose. The roughest kind of calculation from in vivo data demonstrates that one molecule of insulin promotes the extra utilization of some 10^8 - 10^{10} mols of glucose per minute of activity. It would seem obvious therefore that one should look for an influence of insulin on some strongly inhibited limiting process in the course of sugar utilization.

There is no need to retell in detail the physiological and biochemical work in this field beginning with the classical papers of Best, Dale, Holt and Marks in 1926.[4,5] Suffice it to dwell on some of the general conclusions which it was possible to make by 1948.[6-10]

1. Insulin seemed to promote all the known pathways of glucose disposal and transformation, i.e. glycogen storage, fat formation, total oxidation, the shunt system, etc.

2. Not all tissues of the mammalian organism required insulin for their maximal glucose utilization, even though the enzymatic machinery for carbohydrate transformation seemed practically identical in all animal cells (e.g., brain, r.b.c., etc.).

3. It seemed logical to suppose that the effects of insulin were exerted at the very beginning of the scheme for the chemical transformation of glucose, if its influence were to be felt in all the branchings of the metabolic road.

4. No unequivocal, consistently reproducible effect had been obtained on an isolated system in vitro. Insulin had not been shown to act as an activator, as an inhibitor or as a cofactor in any particular isolated relevant enzyme system.

As biochemists reckon time, in the antique period of 1949, it was somewhat hazardous to suggest that insulin may exhibit its effects by exerting an action on a structural cell element, the membrane, which was not known to be a part of the intermediary scheme of metabolism. This was so despite the writings of Rudolf Peters[11,12] on the role of the cytoskeleton, and of the work of Stadie,[13,14] which suggested the need for intactness of the cell in order to obtain insulin effects. It becomes therefore relevant now to review our experiments done between 1949 and 1954.[15-21]

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The hypothesis underlying this work was that insulin may act as a regulator of the rate of glucose flow through the cell membrane to the enzyme apparatus, rather than that this hormone affects reaction rates at steps in the intracellular enzyme array.

Our approach to the problem was to attempt a sharp distinction between movement of a sugar from one position to another and any subsequent metabolism of that carbohydrate. For glucose, this presented a variety of problems involving either deduction from the behavior of trace amounts of radioactive glucose or the use of enzyme poisons. Instead, we chose initially to study the behavior of sugars closely related to glucose in structure but for which enzymes are sharply circumscribed. The eviscerated-nephrectomized animal has long been a tool of the metabolic laboratory. Deprived of the liver, intestines, and kidneys, the preparation can survive for many hours or even days with appropriate care. Many areas of function have been extensively studied in this preparation, showing excellent preservation; other functions are acutely lost with extirpation of these viscera. The metabolism of a variety of substances is limited to the liver, intestines, and kidneys. Included amongst these materials are a variety of sugars. The congener of glucose, galactose, when injected into the eviscerated-nephrectomized dog is no longer metabolized or excreted (figure 1). It behaves as an inert material essentially similar to the behavior of urea and creatinine. When a measured amount is introduced intravenously, the blood concentration shows a descending curve for a time until some volume of distribution of galactose is reached and the concentration remains constant (figure 2). The galactose can now be said to be occupying a space, a volume of water distributed through the body which is available

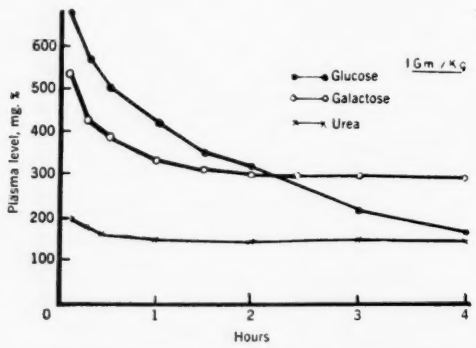

FIG. 1. The glucose curve is typical of the behavior of a utilizable substrate. Urea typifies the distribution characteristics of a nonutilizable material. Galactose is seen to behave in a manner typical of nonutilizable substances.[18]

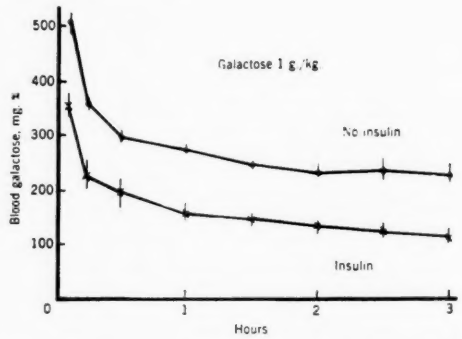

FIG. 2. Galactose was injected at 0 time. Note that insulin caused a faster and wider distribution of the galactose. The galactose "space" in the absence of added insulin was about 45 per cent of body weight; in the presence of added insulin this space widened to about 70 per cent of body weight. The vertical lines indicate range of variation of all values obtained.[16]

to the sugar. We were able to show that the size of this galactose space was a function of insulin. In the absence of added insulin, galactose would occupy about 40 to 45 per cent of the carcass weight. This represented the extracellular fluid space plus the intracellular compartment of certain cells. At any point the addition of insulin resulted in a sharp fall in the blood galactose with the re-establishment of a new equilibrium, representing a distribution space now of 65 to 70 per cent of the carcass weight (figure 3). Insulin made a space available for the entry of galactose which corresponded to total body water (figure 2). Having once achieved this widest distribution, additional insulin promoted no further decrease in blood galactose. Carcass analysis revealed that the movement of galactose from the extracellular posi-

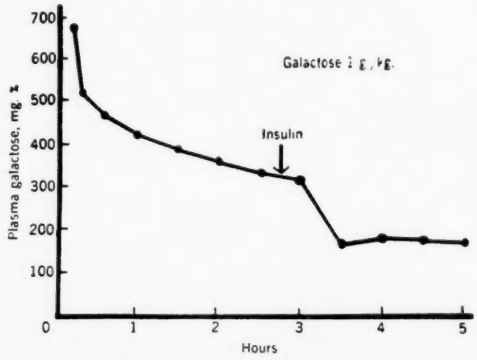

FIG. 3. Galactose was injected at 0 time. The injection of insulin was begun two hours later and continued for the remainder of the experiment. Note that the blood galactose level fell sharply, reached an approximately 70 per cent body weight distribution, and was then maintained.[10,21]

tion to the intracellular compartment previously unavailable without insulin was achieved without any metabolism of the sugar. One could recover the injected material regardless of the area of distribution of galactose or the addition of insulin (figure 4).

Although this demonstration argued by analogy, it appeared as a most satisfactory scheme of the manner by which insulin regulates the metabolism of glucose. It was proposed that certain cells, presumably those of

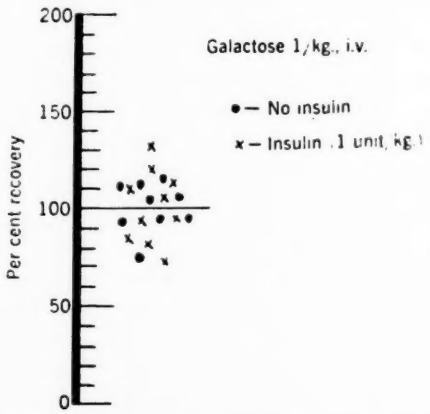

FIG. 4. Note that the figures for recovery of the injected galactose are randomly distributed around the 100 per cent level, for both the insulin and noninsulin group. Average recovery: noninsulin group 103 per cent ± 13 (S.D.); insulin group 100 per cent ± 19 (S.D.).[68] Within the limits of the methods employed galactose is not utilized, in the presence or absence of insulin.[16]

striated muscle and adipose tissue primarily, were denied free and ready access to the entry of glucose. The intracellular apparatus was capable of very high orders of glucose metabolism with the rate of entry constituting the limiting step. Insulin, in some manner, was conceived as facilitating the movement or transport of the sugar across the cellular surface barrier from its extracellular position to the intracellular compartment. For galactose in the eviscerated-nephrectomized preparation this was shown by the manner in which insulin made available a larger space of distribution. Since no further metabolism of galactose was possible, a new equilibrium at a lower concentration in the blood because of wider distribution was the end result. For glucose, the greater rate of entry into the cell would result in a greater rate of its metabolism by the intracellular enzymes.

A variety of sugars was tested in this fashion. The sugars employed fell into two categories. Both hexoses and pentoses, which exhibited the same chemical configuration of hydrogens and hydroxyls about carbons 1, 2 and 3 as seen in the glucose molecule were affected by insulin in promoting their intracellular transport (figure 5). Those sugars which were modified at these carbon positions were indifferent to the presence or absence of insulin (figure 6). The insulin-stimulated transport apparatus was pictured as capable of accepting glucose by virtue of its chemical structure. Sugars of very similar configuration were coincidentally able to ride the

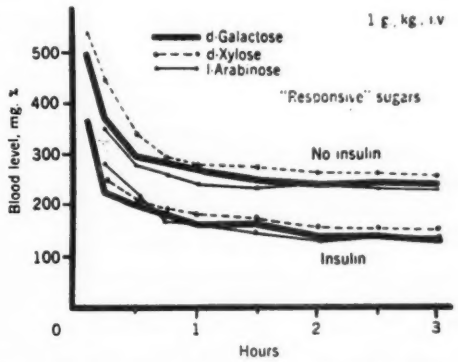

FIG. 5. Effect of insulin on distribution of sugars in eviscerated-nephrectomized dogs. Data previously reported for the effect of insulin on distribution of d-galactose are represented by heavy lines. Note that d-xylose and l-arabinose exhibit identical behavior. For all three sugars an equilibrium is established, in the absence of insulin in a body space of about 40 per cent to 45 per cent. The presence of insulin promotes a distribution of these "responsive" sugars throughout 65 per cent to 70 per cent of body weight, i.e., total body water. All curves are the averages of four to six animals.[19,21]

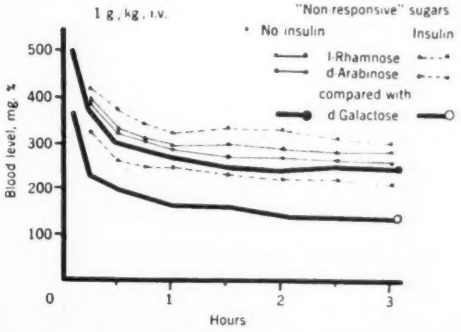

FIG. 6. The effect of insulin on the distribution of sugars in eviscerated-nephrectomized dogs. The behavior of l-rhamnose and d-arabinose is compared with the previous data for d-galactose as in figure 10. By contrast, insulin fails to promote a wider distribution of these "nonresponsive" sugars, and final distributions are established in 35 per cent to 45 per cent of body weight, irrespective of the presence or absence of insulin.[19,21]

transport system into the interior of the cell and afford the experimental approach described above. The stereospecificity found by us, mainly in the dog, does not reflect the picture accurately when other species or tissues are examined. Many naturally occurring as well as synthetic sugars have been tested in this fashion. The configuration of the sugars acceptable to the insulin-stimulated glucose transport system appears to go beyond the initial finding of the glucose model. Considerable manipulation about carbons 2 and 3 is possible without loss of response to insulin. These variations have been reported in different species and in different isolated tissues.[22–25] They may very well reflect the fact that the actual transport apparatus is intrinsically independent of insulin itself. For other cells, there is great species and tissue specificity as to the transport systems for sugars and other substances. Selective entry of materials is a property of organized cells whether of free living micro-organisms or of the tissues of multicellular animals. Even closely related species may exhibit different properties of red cell uptake of sugars.[26,27] When a systematic comparative study of the chemical configuration requirements of the sugar transport system under discussion is undertaken covering the usual laboratory animals and man and the various tissues employed in research, we may very well have to view insulin as activating the transport system in some fashion, but not determining its precise intrinsic properties. One can view the transport system as a specific property of cell structure, normally held in an inhibited state and activated by the hormone, insulin.

The presence in many tissues of a specific transport system for sugars is shown by the facts that the kinetics of sugar entry demonstrates quickly a point of saturation, as well as competitive inhibition between the transportable sugars.

Confirmatory work of the "translocation" theory of insulin action came from many workers employing a variety of in vivo and in vitro technics.

The rat diaphragm,[28,28] the isolated perfused heart[29,30] and the anterior chamber of the eye,[31,32] became tools in the study of the translocation of sugars under the action of insulin (figure 7). The work of Park and his associates[26,33–35] gave particular strength to the glucose transport theory of insulin action by directly demonstrating the phenomenon for glucose in a manner analogous to that seen with the nonutilizable sugars. They argued that even though the amounts of free glucose inside the cell were small, they could be used to distinguish between an action of insulin on permitting glucose entry rather than an action promoting increased enzymatic dissimila-

	Fasting blood glucose (mg./100 ml.)	$10^2 k_{in}$ mean	#
A—Animals in hypoglycemia phase	60–120	6. 4 ± 0.49	5
B—During phase of rising blood sugars	150–330	1. 6 ± 0.11	7
C—Animals with established hyperglycemia	475–580	1.02 ± 0.10	6
D—Normal rabbits		1.99 ± 0.36	10

[a]Taken from the review by Stadie

FIG. 7. Rate of penetration of glucose (k_{in}) across blood-aqueous barrier (rabbit's ciliary body) following intravenous injection of alloxan correlated with the fasting blood glucose concentration[a]. Data from Ross.[34,35]

tion. If insulin promoted increased entry of glucose then there should be an increase in free intracellular glucose (figure 8). However, if insulin operated by virtue of directly promoting a greater rate of utilization of glucose by the enzymes, then the free intracellular glucose should diminish and thereby allow further glucose entry as a secondary consequence. Using a number of conditions involving both excess loading with glucose and lowering the temperature of the tissue, these workers were able to show that one of the consequences of exhibition of insulin was a rise in the free intracellular glucose, clearly indicating a primary role of insulin in permitting more glucose to enter the cell. Glucose behaved in a manner quite parallel and analogous to that of galactose. Rapid phosphorylation by hexokinase carried the glucose off into intermediate pathways, maintaining a continuous flow of the sugar into the cell in this fashion. The cell's surface was the limiting barrier to the metabolism of glucose, and insulin determined the rate of transport of the sugar through the limiting and confining cell membrane.

Insulin is not the only agent which facilitates sugar transfer. Clinical experience and the more recent ex-

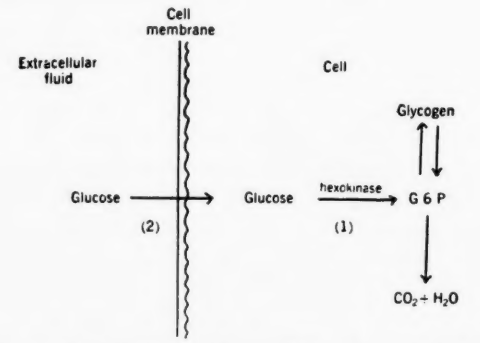

FIG. 8. Schema to illustrate the possibility of a reaction at the cell surface which precedes the first phosphorylation of glucose and is concerned with the transfer of the sugar into the cell interior from the extracellular compartment.

perimental work of Ingle[36] have shown that glucose utilization is increased and the blood sugar is sharply reduced during muscular exercise in the complete absence of insulin. Muscular work leads to the transfer into the cell of sugars which are themselves not utilized. In other words, muscular work influences the cell surface system in the same manner as does insulin (figures 9 and 10). It is suggested that this influence is "humoral," because stimulation of one muscle group of a hind limb leads to the distribution of galactose in the total volume of body water, and because denervation of the exercising limb has no influence on this distributive action.

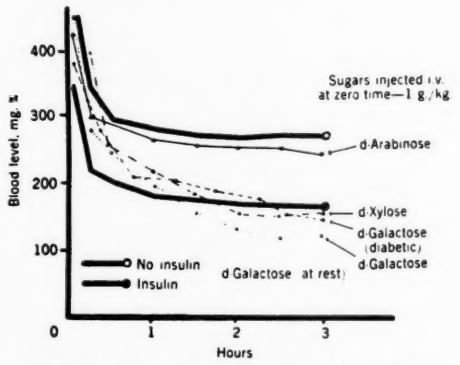

FIG. 9. Distribution of sugars in working eviscerated-nephrectomized dogs. The distributions of sugars found to be "responsive" and "nonresponsive" in peripheral rat tissues are tested here in the dog and compared with the previously reported action of insulin on the distribution of d-galactose. Muscular work promotes wider distribution of d-xylose and d-galactose just as insulin does, but fails to influence the behavior of d-arabinose, which is nonresponsive to insulin. This insulin-like action of muscular work is just as effective in the chronically depancreatized preparation and is thus independent of insulin.[20,21]

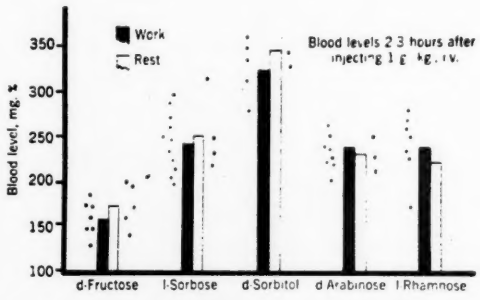

FIG. 10. The effect of muscular work on the distribution of sugars in eviscerated-nephrectomized rats. These sugars are again nonutilizable and the final blood levels at two to three hours represent complete distribution of the test sugar. By contrast with the results illustrated in figure 9, the distribution of these substances is unaffected by muscular work. The various "nonresponsive" sugars achieve different body distributions or "spaces," but in each instance they are the same at work as at rest.[20,21]

Goldstein[37,38] has recently shown that the insulin-like effect of muscular work can be transferred from a working donor animal to a resting recipient by means of cross-circulation. It is generally agreed that glucose entry is facilitated by muscular work, but the data of other laboratories interpret this effect as being restricted to the working parts only.[39-41]

If a tissue is injured, as occurs when a rat diaphragm is cut at the insertion into the ribs, or it is kept in vitro for a prolonged period, the rate of sugar transport is enhanced and the insulin effect is diminished.[23]

Randle and his coworkers[28,42] have shown that oxygen lack, and drugs which are known to inhibit oxidative processes, enhance the uptake of glucose or of xylose by rat muscle in vitro. The phenomenon is reversible by correcting the tissue anoxia.

These nonhormonal effects which simulate the action of insulin on sugar transport are in favor of the supposition that the rates of transfer are kept restricted by some material or condition, which is removable by insulin; by muscular work and by the results of tissue anoxia.

Park et al.[35] found that the enhancement of sugar transfer by insulin was bidirectional. The rate of efflux as well as of influx was greater in the presence of insulin. This work on the perfused heart was confirmed by Goldstein in the eviscerated dog.

The above considerations and related data permit us to list some of the characteristics of the sugar transport systems:

1. In most tissues glucose intake is not "active," i.e., there is no accumulation against a gradient. In the intestinal tract (and probably the renal tubule) membrane transport is transformed into an "active" type by the presence in those cells of a separate "accumulating" system.[44]

2. Stereospecificity is a feature of the system, pointing to specific carriers or structural conditions.

3. Only the transport systems of some tissues (muscle, heart, fat cells, fibroblasts, etc.) are activated by insulin. In other organs (brain, gut, kidney, RBC etc.) glucose transfer rates are not enhanced by the hormone.

4. The influence of temperature on the insulin effect is not very significant. Q_{10} for this action is about 1.0-1.2.[21,45]

5. The more nearly "intact" and well oxygenated an "insulin sensitive" tissue is maintained, the slower is the rate of its glucose transport and the greater is the

enhancing effect of the hormone.

The so-called hormonal antagonists to insulin do not seem to exert their action at the locus of transport. They seem rather to inhibit steps further along the metabolic path and thus to impose a brake on the rate of over-all sugar transformation.

Park[35] and Kipnis[23] and their respective coworkers, as well as our laboratory,[46] have shown that epinephrine inhibition of glucose utilization is exerted by its effect at or near the hexokinase step (due to glucose-6-phosphate accumulation) and not upon the transport system (figure 11).

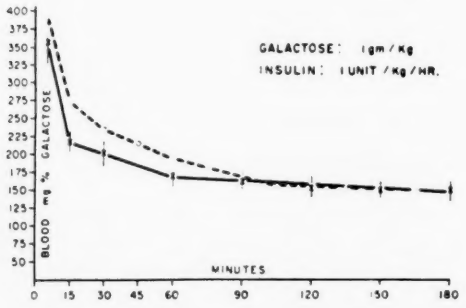

FIG. 11. Solid curve represented the average concentration of blood galactose in insulinized, eviscerated dogs given 1 gm. galactose/kilogram body weight at time 0 and the vertical lines through each represent the range of variations found.[6] Open circles are average results obtained on five dogs on which these experiments were repeated with the variant that 25 μg. epinephrine/kilogram/hour were infused throughout the experiment.[46]

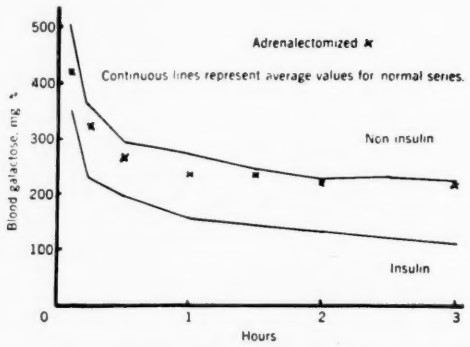

FIG. 12. The eviscerated-adrenalectomized dog exhibited a "normal" galactose distribution despite the insulin sensitivity of the intact animal.[18]

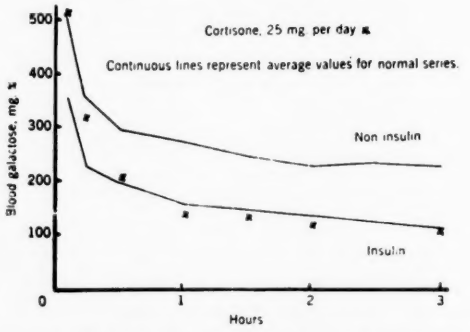

FIG. 13. Insulin resistance exhibited in the cortisone-pretreated animal did not alter the galactose-distributing action of insulin after evisceration.[18]

In the dog in vivo neither total adrenalectomy nor the administration of active glucocorticoids opposes the translocation of galactose under the influence of insulin[18] (figures 12 and 13).

The pituitary growth hormone may itself oppose the transport step[26] but the major effects of this gland are perhaps exerted on some early step in glycolysis rather than on the membrane.[23,47]

Applying Occam's razor, the principle of economy in nature, one may postulate that the plasma membrane of animal cells possesses a basic type of sugar transport system regulating the inflow capacity. Modifiers of this basic system are superimposed in certain tissues. Thus in the intestinal epithelium an "accumulator" is added permitting active transport against the gradient. In muscle, heart, connective tissue, fat cells, etc., the modification seems to consist of an almost complete closure of the system when insulin is absent.

The findings which indicate that in certain tissues (muscle, connective tissues) the transfer system for glucose is ordinarily fairly inactive, and that its rate of action is increased both by insulin and by muscular work, makes good biological sense from the standpoint of the needs of the organism as a whole.[48] During the time when no carbohydrate is being absorbed from the digestive tract, the liver maintains the blood sugar by gluconeogenesis. Over half of the hepatic glucose production is ordinarily used by the brain. If the large extent of cell surface in muscle and connective tissue were at all times open to glucose entry, the blood sugar would soon fall to levels incompatible with normal brain function. When the blood sugar is raised (after a meal), insulin secretion is stimulated. The insulin-sensitive tissues are then "opened" to glucose, which is stored as glycogen and fat for future use without producing hypoglycemia.

Muscular work does not ordinarily raise the blood sugar. Insulin is not mobilized to open the muscle gates for the increased utilization of work. Instead, some factor is released by muscle in amounts proportional to

the performance of work, which has effects similar to insulin. It would be of great interest to search at what point in evolution the muscle factor emerged in relation to the point of appearance of insulin.

Those organs and tissues which operate on "one gear," i.e., those which do not greatly increase or decrease their energy expenditure, have an "open" glucose transfer system; while the working and storage cells, which work on several "gears," seem to have developed devices which open the gates for entry of the important blood-borne metabolite, glucose, when it is needed for work or when it is present in excess in the circulation.

It is evident that although the action of insulin on glucose transport is exerted at the cell membrane, we cannot as yet approach a description of its action in biochemical terms. Several theories have been proposed.

Thus Fisher[49] has suggested that the translocation is effected by a specific carrier, and has calculated that insulin could increase the rate of transfer by enabling the sugar-carrier complex to dissociate more easily (figure 14). No critical test of such a suggestion is yet available.

Barrnett and Ball[50] have observed that insulin stimulates the formation of small vesicles at the cell surface of fat cells. This phenomenon called pinocytosis, is probably the mechanism by which many cells "drink in" exogenous materials. Perhaps sugar is also conveyed in such "containers" to the cell interior (figure 15). At present there are objections to the acceptance of such a mechanism of transport for sugars, namely the exhibited stereospecificity, the need for the simultaneous movement of rather large volumes of water in which the sugar is dissolved, and the absence of stimulation of pinocytosis in muscle cells, despite their sensitivity to insulin.

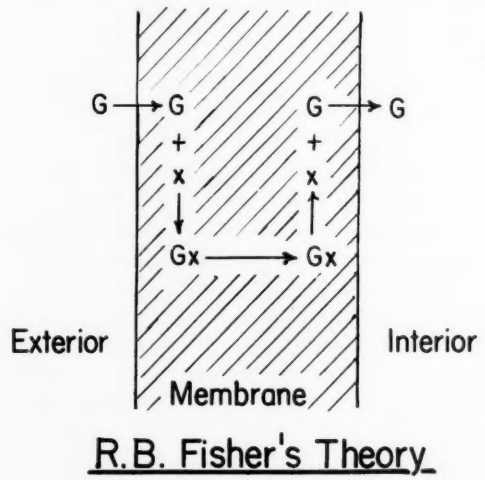

FIG. 14. Insulin is thought to make dissociation of the glucose-carrier complex (Gx) easier. Thus rate of flow of glucose through membrane would be facilitated.

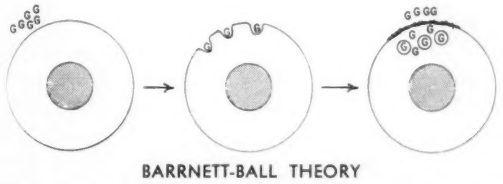

FIG. 15. Insulin is thought to increase formation of vesicles containing glucose in the solution, thereby the glucose is conveyed to the cell interior.[50]

Randle[28] has suggested that a mechanism, requiring continuous oxidative energy (ATP?) exists at the membrane which keeps the surface poorly permeable to glucose. Insulin, anoxia, muscular work, etc., interfere with this system. In their excellent review the Teppermans[51] christened this inhibitory system—the "keeper-outase."

Our own formulation[48,52] is close to that of Randle (figure 16). It postulates that in the insulin sensitive cells the glucose transport systems (GTS) are kept inactive, covered or inhibited by the specific insulin receptor material. Combination of the hormone with its receptor opens the gate temporarily. Resynthesis of the receptor material requires oxidative energy.

The concept of insulin action here presented has the virtue of much experimental support and of being able to account for many of the phenomena which follow the administration of insulin to man or animal.

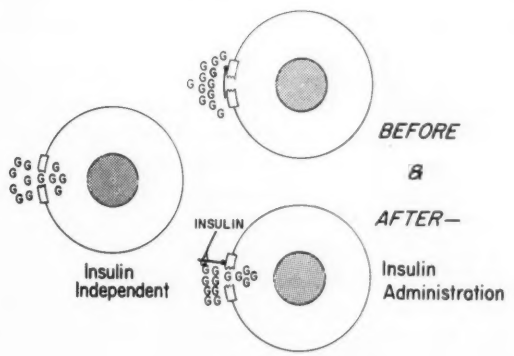

FIG. 16. The transport system is steriospecific for certain sugars. In some cells the system is "covered" or inhibited. Insulin is thought to combine with the "cover" and thus allows the transport to occur.

However, all is not "sweetness and light." We should consider now those experimental data *which do not at present* fit this unitary concept of insulin action.

1. It would seem from the work of Shaw and Stadie,[53,54] from that of J. Larner,[55,56] and from the older in vivo experiments of Soskin and Levine,[57] that insulin may favor the glycogen pathway out of proportion to the other transformations of the glucose molecule. Is there an additional action of insulin on some step in the uridine system, or is the architecture of the muscle cell such that the GTS is linked to the glycogen synthesizing part of the machinery, which leads to channeling of the glucose initially? Glucose entering by diffusion may be more evenly distributed in the cell and thus lead to less glycogen storage. In adipose tissue pathway favoritism does not on the whole obtain.[58]

2. Bessman[59] has shown a positive effect of insulin on glycogen levels of "damaged" diaphragm, at a time when xylose was freely admitted in the absence of insulin. It is difficult to know whether in such preparations the glycogen effect was obtained in the "damaged" or the "undamaged" portions of the tissue.

Chain and co-workers[60] could not obtain an insulin effect on galactose entry into the rat diaphragm. To our knowledge this is the only instance in which the galactose effect was not shown. They contend also that while insulin promoted glycogenesis from glucose it failed to increase the yield of CO_2. Chain concluded that insulin must act on a pivotal reaction in cell energetics and not on membrane transport. The conclusions arrived at by Chain et al. seem too sweeping, considering the large number of workers who have provided evidence for an insulin effect on the transport of nonutilizable sugars. In addition, many of the conclusions are based upon absolute counts of radioactivity in intermediates, without due consideration of their turnover.

3. The effect of insulin in favoring incorporation of amino acids into muscle proteins is observable in the *absence* of glucose in the medium.[61,62] This accords with the in vivo observations of Ingle[63] in the rat but not with our own attempts to see an influence of insulin (without glucose) on the disposal rate of amino acids in the dog. In adipose tissue insulin affects amino acid incorporation into proteins only in the presence of glucose.[64] The action in adipose tissue fits the unitary concept, that on muscle protein synthesis does not.

4. The presence as well as the nature of the effects of insulin on metabolic parameters of the liver remain curiously contradictory and difficult to resolve. Unlike most mammalian cells the parenchymal hepatic cell is leaky and sterically nondiscriminatory. Glucose transport is nonlimiting and free sugar is found in the cell interior.[65] An insulin effect would have to be of a type different from that on muscle or fat tissue, unless a GTS exists at an interface *within* this cell.

On the positive side, arguing *for* an insulin effect are the isotope experiments of Weinhouse[66,67] and the work of Madison[68,69] on Eck-fistula animals. These indicate inhibition of liver sugar output or increased liver sugar uptake. Such results may be contrasted with the work of de Bodo's laboratory[70–72] and the measurements of Mahler, Shoemaker et al.[73,74] However, de Bodo et al. find a curious braking effect of insulin preventing the increased sugar output which follows hypoglycemia. Hetenyi, Wrenshall and Best[75] have found some effect on liver sugar output in normal, but not in depancreatized animals.

Direct effects on isolated liver have been sought for many years and some have been found. Recent examples are the work of Miller and Haft;[70,77] of Penhos and Krahl[78] and of Mortimore.[79]

Let us for the moment assume the existence of direct effects of insulin on liver metabolism. One could still hold to a unitary concept of action by assuming that insulin combines with a specific chemical group. This combination at certain membranes permits freer entry of sugar. A similar reaction in the cell interior may result in effects other than glucose transport. It is *possible* to think in this manner, but it may not be profitable at this juncture, before agreement is reached about the effects themselves. Gaps and contradictions still block our way.

It was of course to be expected that the glucose transport theory of insulin action would lead to a host of new problems and a great need for further work. Among such problems are:

1. The chemical make-up of animal cell membranes.

2. The architectural relationship of the cell membrane to the organized enzyme packets of the cell (reticular network, mitochondria, microsomes, etc.).

3. Is glucose transport the property of the membrane as a whole, or are there specific loci or patches which constitute the active system?

4. What roles does insulin play in regulating the output of the anterior pituitary hormones? The gland is insulin responsive and removal of the gland tends to normalize the metabolic deviations in the diabetic liver.

5. What is the nature of GTS which would explain as well, its actions on larger molecules with *glucose* endgroups [cardiac glycosides,[80] dextran,[81] ovomucoid[81]]? It has been shown that insulin facilitates cell entry of

such substances into heart muscle and mast cells, respectively.

These are only a few of the problems which exist. We hope to devote ourselves to some of them; we are in the midst of work on others.

The mechanism of insulin action on a molecular level remains unknown; its further elucidation would seem to depend upon a better knowledge of the structural biochemistry of cells. One major site of action seems however to be established in a firm fashion, at the cell membrane of certain tissues.

The original discovery by Banting and Best had its practical, therapeutic effect immediately. Its basic, academic ramifications are by now myriad, and they are ever widening in volume and extent. All of us in the field will, I hope, continue to add our own small twigs to this flourishing tree.

SUMMARIO IN INTERLINGUA

Le Question del Mechanismos per Que Insulina Exerce Su Effecto: Discurso Memorial Banting

Le discoperta original de insulina per Banting e Best produceva immediatemente importantissime effectos in le practica therapeutic. Quanto al ramificationes theoric, i.e. de recerca basic de ille discoperta, illos continua—ancora hodie—devenir de plus in plus complexe.

Le presente discurso discute specificamente le question del mechanismos per que insulina exerce su effecto. Le discussion es organisate historicamente. Illo summarisa le contributiones facite per le autor e su collaboratores in le curso del passate dozena de annos sed representa simultaneemente un revista del litteratura.

In terminos general, le effecto de insulina es un resultato de su activitate catalytic. Minimissimemente micre quantitates del hormon effectua le augmentate utilisation de vaste quantitates de glucosa.

Le discussion del hypothese general que guidava le autor in su recercas es precedite per le formulation de certe conclusiones que, jam in 1948, pareva adequatemente documentate. Istos es:

1. Insulina pareva promover le activitate in omne le cognoscite cyclos metabolic afficiente le elimination e transformation de glucosa, i.e. le magasinage de glycogeno, le formation de grassia, le oxydation total, le systema del shunts, etc.

2. Non omne le tissus del organismo mammalian requireva insulina pro le utilisation maximal de glucosa, ben que le mechanismos enzymatic pro le transformation de hydrato de carbon pareva practicamente identic in omne le diverse cellulas animal (per exemplo le cellulas del cerebro, le erythrocytos, etc.).

3. Il pareva logic supponer que le effectos de insulina esseva exercite al prime comenciamento del curso del transformation chimic de glucosa, viste que su influentia esseva presente in omne le brancas del via metabolic.

4. Nulle inequivoc e uniformemente reproducibile effecto de insulina habeva essite obtenite a ille tempore in un isolate systema in vitro. Il non habeva essite possibile monstrar que insulina age como activator o como inhibitor o como cofactor in un specific systema enzymatic in isolation.

Le hypothese mesme que le autor postulava alora como base de su labores futur supponeva que insulina age como regulator del volumine e celeritate del fluxo de glucosa a transverso le membrana cellular ad in le apparato enzymatic intra le cellula e que illo non affice le reactiones representante le diverse stadios del processos enzymatic intracellular. Methodologicamente isto significa que un stricte distinction esseva mantenite inter (1) le movimento, i.e. le transporto de un sucro ab un sito al altere e (2) le subsequente metabolismo experientiate per illo.

Le theoria del mission de insulina como regulator del passage de sucros per le membrana cellular es analysate in grande detalio. Le investigationes del autor e su gruppo e de alteres es summarisate criticamente in tanto que illos supportava le theoria e etiam in tanto que illos pareva arguer contra ille theoria.

Le investigationes continua. Le areas in que nove recercas es urgentemente requirite pro clarificar le situation additionalmente include le sequentes:

1. Le constitution chimic del membranas de varie cellulas animal.

2. Le relation architectural inter le membrana cellular e le organisate conjuncto de enzymas in le cellula, incluse le systema reticular, le mitochondrios, le microsomas, etc.

3. Le question de si le transporto de glucosa es un proprietate del membrana cellular in su integritate o si il existe locos o areas specific que representa le active systema de transportation.

4. Le question del rolo de insulina in le regulation del rendimento de hormones antero-pituitari.

5. Le question del aspecto particular del systema de transporto de glucosa que explicarea le facto que illo accepta non solmente glucosa per se sed etiam plus grande moleculas con gruppos terminal de glucosa, como per exemplo le glycosidas cardiac, dextrano, etc.

In conclusion le autor nota que, ben que le mechanismo del action de insulina al nivello molecular remane incognoscite (dependente in su elucidation additional de meliorate cognoscentias del biochimia structural del

cellula), un sito major del action de insulina pare esser firmemente establite: le membrana del cellulas de certe typos de tissu.

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The Hyperlipemic Effect of a Low-fat, High-carbohydrate Diet in Diabetic Subjects

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Low-fat, high-carbohydrate diets, until recently avoided in the treatment of diabetic patients, have been shown to lower serum cholesterol levels and reduce the severity of vascular complications, while improving glucose tolerance and lowering insulin requirements.[1-5]

However, increases in the concentration of plasma triglyceride (hyperlipemia) have been noted in nondiabetic subjects during the administration of low-fat, high-carbohydrate diets. Many of the patients with essential hypertension who had been maintained on rice diets (85 to 95 per cent carbohydrate) for several months developed a "neutral fat" lipemia.[6,7] Increased plasma tritriglyceride levels have been observed in normal subjects[8] and patients with coronary artery disease[9] during the feeding of low-fat, liquid formula diets. Increases in plasma low density lipoproteins (S_f 100-400) were also noted in normal subjects on low-fat diets.[10] A major proportion of patients with essential hyperlipemia became more markedly lipemic when carbohydrate was isocalorically substituted for fat in the diet.[11]

The lipemic effect of these low-fat, high-carbohydrate diets may, however, be only temporary. In a recent study of the long-term effects of reduction of dietary fat calories in South African white and Bantu prisoners, it was observed that serum triglyceride levels returned to normal after several months.[12] Elevated levels in patients with essential hyperlipemia, maintained on rice diets, also returned toward control values after three months.[13]

Previous observations in diabetic subjects appeared to indicate that hyperlipemia was not the result of increased dietary fat levels;[14] indeed, amelioration of lipemia during treatment with high-fat diets had been reported.[15,16] The more recent evidence linking hyperlipemia with the administration of low-fat, high-carbohydrate diets raised the question of whether insulin-dependent diabetics would respond similarly.

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METHODS

Patients with severe adult or juvenile diabetes, without renal or hepatic disease, served as subjects. Six studies were performed in five patients on a metabolic ward. They received diets kept at constant caloric levels designed to maintain body weight. Insulin dosages were also kept constant whenever possible. Two diets were compared: (1) a basal formula, containing 15 per cent protein calories, 40 per cent fat calories as corn oil, and 45 per cent carbohydrate calories, fed as a liquid diet; (2) a low-fat, high-carbohydrate diet, containing 15 per cent protein calories, 85 per cent carbohydrate calories, and virtually no fat, fed as a liquid diet with rice and matzoh (unleavened bread) supplements, for periods ranging from two to ten weeks. The subjects received supplementary multivitamins, iodized salt, and ferrous gluconate.

Samples of venous blood were obtained after an overnight fast prior to the administration of insulin. Glucose was measured in filtrates of whole blood[17] using the enzymatic reagent Glucostat (glucose oxidase, horseradish peroxidases, phosphate buffer, and o-dianisidine; Worthington Biochemical Corp., Freehold, New Jersey), as described by Saifer and Gerstenfeld.[18] Plasma nonesterified fatty acids were determined by the single extraction method of Dole.[19] Measurements of total cholesterol[20] and lipid phosphorus[21] were made on aliquots of serum.

Triglyceride analyses were performed by a modification of a procedure previously described.[22] A 1 ml. aliquot of plasma is extracted in 20 ml. chloroform-methanol (2:1 V:V), filtered into a glass-stoppered, narrow-necked bottle graduated at 25 ml., and made up to volume. Five ml. water is added, and the two phases allowed to separate by standing overnight at 4° C. The upper aqueous phase is removed by gentle suction and the chloroform phase brought to a final volume of 25 ml. with additional chloroform-methanol (2:1 V:V). Aliquots of this extract are dried and then redissolved in petroleum ether (BP 30°-60°).

Lipids in the petroleum ether extract are fractionated

by a modification of the procedure described by Hirsch and Ahrens[23] using batch elution.[24]

An aliquot of the petroleum ether extract is shaken several minutes with 0.5 gm. activated silicic acid (Bio Rad Laboratories, Berkeley, California). The petroleum ether is then removed and discarded by suction through a glass-wool-plugged glass adaptor placed into the silicic acid slurry. The silicic acid is next eluted with anhydrous ethyl ether (approximately 3 ml. shaken with silicic acid followed by three ether washes). This fraction contains glycerides, free of phospholipids and interfering substances. After evaporation to dryness it is saponified and extracted by the Carlson method,[26] modified as follows: 1 ml. of freshly prepared 0.4 per cent of KOH in 95 per cent ethanol is added to the samples, to a tripalmitin standard (approximately 0.5 μM), and to a blank tube. The glycerides are hydrolyzed at 65° C. for thirty minutes. Following cooling, 2.0 ml. water, 0.1 ml. 10 N H_2SO_4, and 4 ml. of ethyl ether are added to each tube. The phases are mixed well, allowed to separate, and the upper phase carefully removed and discarded. One ml. aliquots of lower phase are transferred into flasks for determination of glycerol;[26,27] 0.5 ml. .05 M sodium metaperiodate is added, and the reaction allowed to proceed ten minutes at room temperature. Then 0.5 ml. 0.5 M sodium arsenite and 3.0 ml. water are added and the flasks thoroughly mixed. Duplicate 1.0 ml. aliquots are transferred into 100 x 13 mm. tubes, and 10 ml. of 0.2 per cent chromotropic acid reagent is added. The tubes are capped loosely, mixed thoroughly and heated in a boiling water bath away from direct light for thirty minutes. After cooling, optical densities are read at 570 mμ.

Total urine collections were made, preserved under toluene and refrigerated. Aliquots were analyzed for reducing substances by titration with copper sulfate,[25] and for total organic acids by the method of Van Slyke and Palmer[29] modified for use with a *p*H meter.

Paper electrophoresis was performed with RSCO Model E-800-2 apparatus (Research Specialties Co., Richmond, Calif.) on Whatman No. 3 MM paper in Veronal buffer, *p*H 8.6, ionic strength 0.06. Aliquots of plasma (approximately 0.05 ml.) were applied to a free hanging filter paper strip (3.5 x 54 cm.) and a constant voltage of 200 volts applied for sixteen hours at room temperature. The strips were dried in air and stained for lipoproteins with Fat Red 7 B (Ciba) according to the method of Straus.[30] Densitometric measurements of the lipid stained strips were made on a Photovolt recording densitometer using a 525 mμ filter.

The removal rate of particulate fat from plasma was measured in one subject during each of the two dietary periods. A trace quantity of C^{14}-labeled tripalmitin (7-11 μc) was complexed with the patient's plasma in vitro, generating labeled "chylomicrons" (particle size <800 mμ), and given intravenously as a single rapid injection. Blood samples were obtained from an indwelling needle at one-minute intervals for the first ten minutes, and at two- to ten-minute intervals thereafter. Plasma samples were extracted in chloroform-methanol (2:1 V:V), and, after filtration, drying, and re-extraction in 5 per cent ethanol-95 per cent ethyl ether, passed slowly through columns of freshly charged Amberlite IRA-400 ion exchange resin to remove fatty acids.[31] Eluates were assayed for radioactivity with a Packard TriCarb automatic scintillation counter using 2,5-diphenyloxazole and p-bis 2-(5-phenyloxazolyl)-benzene as phosphors.

RESULTS

On the high-carbohydrate, low-fat diet, plasma triglyceride concentrations increased two- to fourfold (p <0.01) in five of the six studies (figure 1) (table 1). This increment became evident during the first week and reached maximum levels in one to four weeks. (The patient who did not show this response was a seventeen-year-old male juvenile diabetic who was also atypical in that he gained weight (0.1/kg./day) and demonstrated increased glycosuria throughout the six weeks of high carbohydrate feeding). In two patients (OD; JW) following a rapid initial response, a tendency to return toward control levels was noted (figure 2). This tendency was defined more clearly in a study on one patient (FF II) who was maintained on the test diet for ten

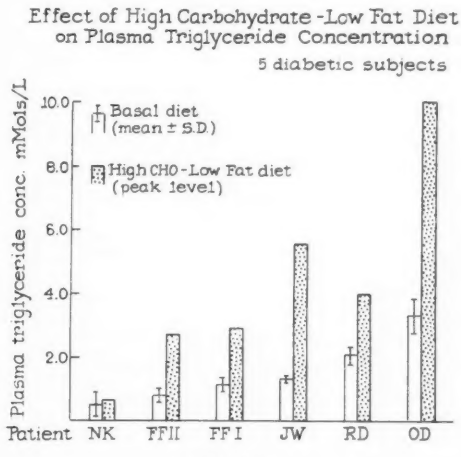

FIGURE 1

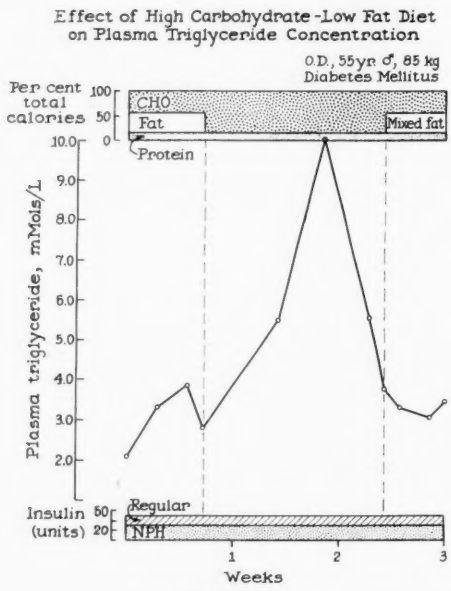

FIGURE 2

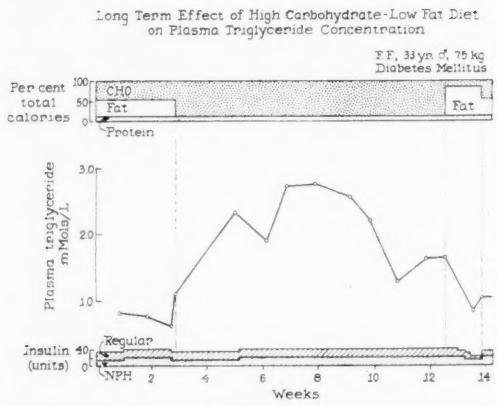

FIGURE 3

weeks (figure 3). After seven weeks, triglycerides were significantly reduced from previous levels ($p<0.02$), but remained higher than control values ($p<0.05$). In all studies, return to the basal diet promptly restored baseline triglyceride concentrations.

Small increases in phospholipid levels were observed ($p<0.05$; paired comparisons) paralleling the elevations in triglyceride. Serum cholesterol levels remained unchanged ($p>0.10$) (table 1).

Plasma turbidity increased during the high-carbohydrate, low-fat period. Filter paper electrophoresis of the plasma showed a rise in the proportion of lipoprotein remaining at the origin ($p<0.02$) (figure 4), including the patient (NK) in whom increased plasma triglyceride concentration could not be demonstrated (table 2). One previously untreated diabetic (RD), who had a marked hyperlipemia (triglycerides, 92 mM/L.; normal range, 0.6-1.7 mM/L.)[22] that responded to insulin therapy prior to the study, maintained a high proportion of origin lipid on the basal diet, although plasma triglyceride concentrations were near normal. In this case, no shift in lipoprotein distribution was apparent.

Despite the increased load of dietary carbohydrate, insulin requirements were not raised, as reflected by unchanged fasting blood glucose and plasma fatty acid levels, urinary sugar and organic acid excretion.

On the low-fat, high-carbohydrate diet, the removal rate of a trace dose of C^{14}-labeled tripalmitin during the first ten minutes after rapid intravenous administra-

TABLE 1

Subject	Age	Sex	Wt. (kg.)	Daily insulin dose (units)	TRIGLYCERIDES Basal Mean* mM/L.±S.D.	(n)	High carbohydrate Max. Level mM/L.	% ↑	p†	PHOSPHOLIPIDS Basal Mean mg%±S.D.	(n)	High carbohydrate Mean mg%±S.D.	(n)	p	CHOLESTEROL Basal Mean mg%±S.D.	(n)	High carbohydrate Mean mg%±S.D.	(n)	p
FF I	33	M	75	50	1.18±.20	(5)	2.96	151	<.002	170±0	(3)	213±9	(3)	<.01	157±9	(3)	164±17	(4)	N.S.
FF II	33	M	75	40	.81±.21	(4)	2.75	240	<.01	133±15	(2)	225±40	(7)	<.05	107±6	(2)	169±24	(7)	<.02
JW	47	F	56	60	1.34±.08	(4)	5.53	313	<.001	219±26	(2)	291±2	(2)	N.S.	150±11	(2)	157±7	(2)	N.S.
OD	55	M	85	50	3.30±.53	(3)	9.96	202	<.02	297±5	(2)	344±54	(2)	N.S.	205±11	(2)	219±24	(2)	N.S.
RD	21	M	65	50	2.07±.26	(3)	4.00	93	<.05	183±3	(2)	204±17	(3)	N.S.	140±23	(2)	125±12	(3)	N.S.
NK	17	M	46	45	.54±.40	(3)	.83	54	N.S.	154±37	(2)	143±9	(7)	N.S.	95±3	(3)	111±6	(7)	<.01

*Standard deviation
†Probability that differences between values for basal and test periods are significant (N.S. = $p>.05$).

TABLE 2

Effect of high-carbohydrate, low-fat diet on lipoprotein distribution (filter paper electrophoresis)

Patient	Basal diet Per cent of total			High carbohydrate Per cent of total		
	"O"	β	α	"O"	β	α
FF I	16	62	22	53	37	11
FF II	23	66	11	57	37	6
JW	26	54	20	51	32	17
OD	15	70	15	44	45	11
RD	45	47	8	44	49	7
NK	18	43	39	28	50	22

"O" = lipoprotein remaining at origin.
β = Beta lipoprotein.
α = Alpha lipoprotein.

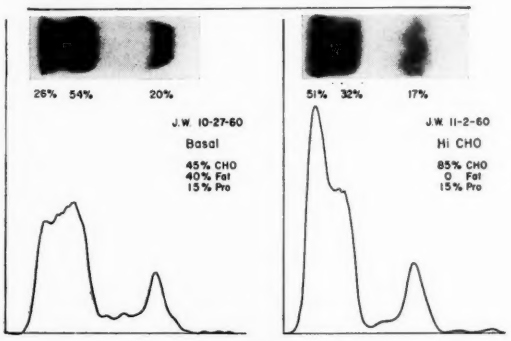

FIGURE 4

tion ($T_{1/2} = 4.1 \pm 0.3$ min.) was similar to that observed on the basal diet ($T_{1/2} = 4.5 \pm 0.2$ min.) when the identical test was performed (figure 5).

DISCUSSION

These results indicate that the insulin treated diabetic is capable of exhibiting a hyperlipemic response to a low-fat, high-carbohydrate diet, similar to that observed in normal, hyperlipemic, and hypercholesterolemic patients. Thus, plasma triglyceride levels in postabsorptive diabetic patients are clearly dependent on antecedent dietary fat and carbohydrate levels.

The relation of this dietary effect to the hyperlipemia with concomitant lipemia retinalis and eruptive xanthoma, occasionally observed in the uncontrolled diabetic, has not been defined in this study. The latter type of lipemia is characteristically more marked, and clears rapidly with insulin therapy. When the one patient who exhibited a massive hyperlipemia that responded

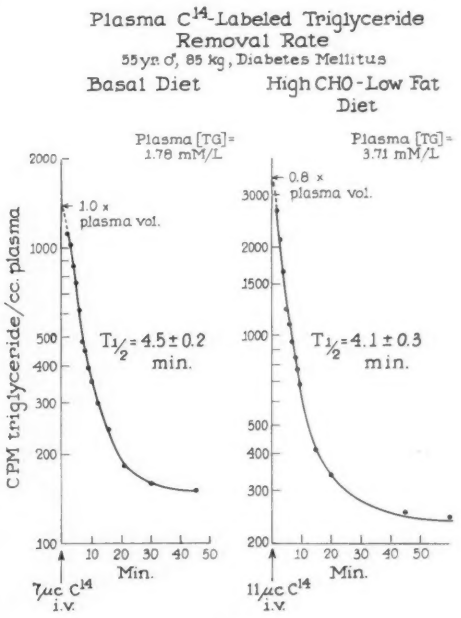

FIGURE 5

to insulin was tested, fasting plasma triglyceride levels doubled in two weeks on the low-fat, high-carbohydrate diet. However, when insulin was omitted while the patient was on this diet, the plasma became milky with a tenfold increase in triglyceride concentration in forty-eight hours.[32]

The possibility that the hyperlipemic response to low-fat, high-carbohydrate feeding may be only transient is suggested by the present data. The earliest complete reversal of the increment in plasma triglyceride concentration observed by Antonis and Bersohn was three months.[12] In this study, three patients exhibited a peak triglyceride level with a subsequent decline in less than two months. More long-term studies are needed, however, further to define this apparent adaptation.

The ability of the diabetic patient to tolerate an extreme increase in the proportion of carbohydrate calories in the diet without raising insulin requirements or showing other untoward effects is confirmed. No reduction of serum cholesterol level was observed in the present study, in contrast to the findings with hypercholesterolemic diabetics.[2,3,6] This discrepancy could be related to the fact that the serum cholesterol had already been lowered by a basal diet in which the relatively unsaturated fat, corn oil, served as the sole source of dietary fat.

The alteration in circulating lipoproteins associated with the additional plasma triglyceride is of interest.

The increase in stainable lipid remaining at the origin on filter paper electrophoresis suggests a rise in the concentration of chylomicrons and very low density lipoproteins.[33] The increments in plasma phospholipids and triglycerides had a molar ratio of about 1:5; this would be compatible with an increase in a lipoprotein of density <1.006 ($S_f>20$).[34] These results are in accord with the ultracentrifugal data reported by Nichols et al.[10] for normal subjects on similar diets.

The mechanism by which a low-fat, high-carbohydrate diet induces hyperlipemia remains obscure. Presumably the effect is not due merely to lack of dietary fat, since low-fat, low-calorie diets reduce the level of low-density lipoprotein.[10] Increasing the proportion of dietary carbohydrate, then, must in some manner either limit the removal or increase the entry of triglyceride-rich, low-density lipoproteins into plasma. The limited data available do not support the first possibility. The C^{14}-labeled tripalmitin "chylomicrons" were cleared at the same rate during basal and high-carbohydrate feeding periods. If native very low-density lipoproteins are also removed at an unchanged rate when the plasma triglyceride pool is increased during high-carbohydrate feeding (figure 5), then the total triglyceride removed from plasma during this period may be increased. Supporting this interpretation, carbohydrate fed rats clear intravenously injected homologous chylomicrons at the same rate as fasted rats.[35,36]

Alternatively, increasing the proportion of dietary carbohydrate may enhance synthesis and esterification of fatty acids, and accelerate their release into plasma as very low-density lipoproteins. Some experimental support for this possibility is derived from studies of lipogenesis in liver and adipose tissue. Carbohydrate feeding enhances the synthesis of fatty acid from glucose,[37-39] and incorporation of fatty acids into glycerides in liver,[40] muscle,[41] and adipose tissue.[42] Insulin treatment of the diabetic may preserve this pathway of glucose utilization, while at the same time rendering him subject to hyperlipemia.

SUMMARY

Insulin treated diabetics exhibited a hyperlipemic response to isocaloric substitution of carbohydrate for fat in the diet similar to that previously observed in normal, hyperlipemic, and hypercholesterolemic subjects. Increases in plasma turbidity, alterations in plasma lipoprotein patterns (filter paper electrophoresis) and small elevations in serum phospholipid levels, suggest that the additional triglyceride in plasma circulates as very low density lipoproteins.

The marked increase in dietary carbohydrate did not raise insulin requirements, as reflected by unchanged fasting blood glucose and fatty acid levels, urinary sugar and organic acid excretion.

High carbohydrate feeding did not appear to alter the clearance of C^{14}-labeled triglyceride from plasma.

SUMMARIO IN INTERLINGUA

Le Effecto Hyperlipemic de un Dieta Povre in Grassia e Ric in Hydratos de Carbon in Subjectos Diabetic

Diabeticos tractate con insulina exhibiva un responsa hyperlipemic al substitution isocaloric de hydrato de carbon pro grassia, simile al responsa previemente observate in subjectos normal, hyperlipemic, e hypercholesterolemic. Augmentos in le turbiditate de plasma, alterationes in le configuration del componentes lipoproteinic in le plasma (studiate per electrophorese a papiro-filtro), e leve augmentos del concentration seral de phospholipido suggere que le triglycerido additional in le plasma circula como lipoproteinas de bassissime densitate.

Le marcate augmento de hydrato de carbon in le dieta non augmentava le requirimentos de insulina, a judicar per le non-alterate valores pro glucosa e acido grasse del sanguine in stato jejun e pro le excretion urinari de sucro e acido organic.

Le dieta ric in hydrato de carbon non pareva alterar le clearance de triglycerido marcate con C^{14} ab le plasma.

ACKNOWLEDGMENT

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Blood Sugar Findings During Pregnancy in Normals and Possible Prediabetics

August Hagen, M.D., Copenhagen

Numerous investigations indicate that women who acquired diabetes after the menopause have had a definitely increased tendency to bear big infants. A prediabetic syndrome in women has been postulated on this basis. Apart from the tendency to bear big infants (with an increased perinatal mortality) obesity appears to be an important sign in prediabetic women, as pointed out by Pirart.[1] The relationship between infant size and the risk of acquiring diabetes has been calculated by Kriss and Futcher.[2] These phenomena are well known and several reviews have been recorded.[3]

It would be reasonable to imagine that the glucose tolerance curves of women who are likely to bear big infants would be abnormal during pregnancy, even though they were normal in the nonpregnant state. Investigations by Basil Jones,[4] Gilbert,[5] John,[6] Kritzer,[7] Lund and Weese,[8] and Jackson[9] indicate that this is so. These investigations, however, were carried out before a possible diabetogenic effect of normal pregnancy had been well defined. Thus, what these authors observed may have been merely the ordinary shift in the glucose tolerance curve during pregnancy.

Glycosuria is the alteration in carbohydrate metabolism during normal pregnancy which has been known longest and is most easily detectable. Since 1856, when Blot[10] reported this observation, numerous investigations have been concerned with fasting blood sugar and glucose tolerance in normal pregnancy, but the results have been conflicting. The findings do not show a definite deviation in the blood sugar during normal pregnancy.

PROCEDURE

A. *Normals (Group A)*

In an effort to elucidate these matters, twenty-eight normal women were investigated. The observations included determination of blood sugar using the Hagedorn-Jensen method, tracing the glucose tolerance curve after oral administration of 1 gm. glucose per kilogram body weight at three, five, seven, and nine months of gestation and once after delivery. During glucose loading, qualitative tests for glycosuria were carried out. Eleven of the subjects also had intravenous glucose tolerance tests (50 ml. of 50 per cent glucose) at the time of the last investigation in pregnancy and postpartum. The women ranged in age from seventeen to twenty-seven years. On the basis of their past history, clinical findings and course of pregnancy they were classified as normal. In particular, the series did not include any patient known to be prediabetic. Details regarding the material and methods have been published.[3]

Table 1 and figure 1 show that during pregnancy the fasting blood sugar is lower, the peak is higher and occurs later, and the blood sugar values at two hours are higher than postpartum. The changes are most marked at seven months, when nine out of twenty-eight blood glucose values had not dropped below 120 mg. per 100 ml. at two and one-half hours. In the last investigation during pregnancy two of the curves were above this normal threshold.

These findings were subjected to statistical analysis by Mr. Arne Nielsen who arrived at the following result, using a "false χ^2 test":

> The fasting blood sugar is lower at seven months than in the other pregnancy investigations, and the latter are lower than those following delivery. All values are significant at the 0.1 per cent limit. The maximum increase, i.e., the difference between the

TABLE 1

Group A. Oral glucose tolerance tests in twenty-eight normal women. Mean blood sugar values at four readings during gestation and postpartum (see also figure 1)

	Months of pregnancy				
Minutes	3	5	7	9	Postpartum
—60	89.1	88.1	83.4	88.3	100.1
—40	87.3	88.6	83.5	86.8	99.2
—20	86.5	89.5	83.8	88.0	100.3
0	88.4	89.5	83.1	89.6	99.5
+20	137.6	131.5	125.9	127.0	143.8
+40	149.3	146.7	151.6	145.9	154.8
+60	139.7	144.3	152.9	145.6	140.4
+80	127.6	133.7	144.1	136.2	126.7
+100	125.6	132.4	137.1	125.5	120.7
+120	119.1	116.0	125.6	112.0	111.4
+140	106.9	105.9	115.4	105.8	104.6
+160	90.3	93.8	103.3	96.2	94.8
+180	88.6	87.9	94.2	84.8	91.6

From Royal Maternity Department A (Professor E. Rydberg, M.D.), University Hospital, Copenhagen, Denmark.

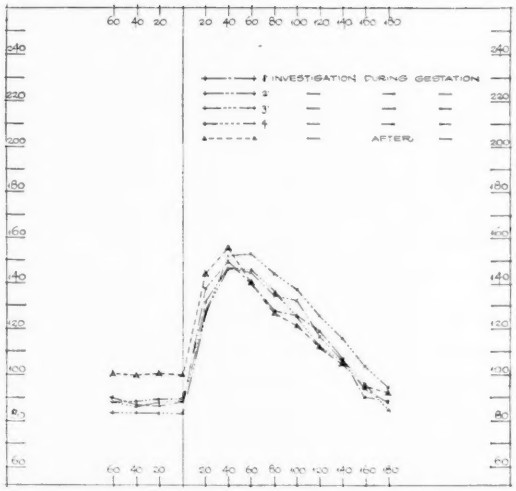

FIG. 1. Mean curves for twenty-eight normal subjects. Fasting blood sugar values and glucose tolerance curves from four investigations during pregnancy and one after. Blood sugar in milligrams per 100 milliliters is shown on the ordinate. Minutes before and after ingestion are shown on the abscissa.

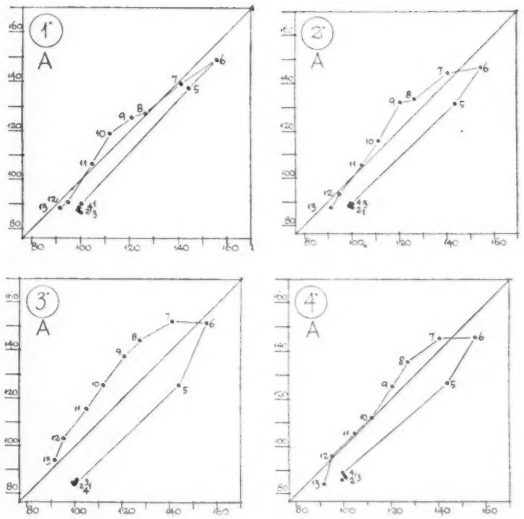

FIG. 2. Mean curves for twenty-eight normal subjects. Blood sugar values at the four investigations during pregnancy (ordinates) are plotted against the postpartum investigations (abscissas) in milligrams per 100 milliliters. 1. at three months 2. five months 3. seven months 4. nine months. See explanation in text.

fasting blood sugar value and the first peak(if this is followed by a decrease of more than 10 mg. per 100 ml. before the next value) is greatest at seven months. This is significant at the 1 per cent limit.

At seven months (1 per cent limit) and less so at nine months (5 per cent limit), the peak after administration of glucose occurs later than at five months.

Lastly, the blood sugar value two hours after glucose is highest at seven months (5 per cent limit) and lowest at postpartum (0.1 per cent limit).

In figure 2 the mean blood sugar values of the four tolerance tests during gestation have been plotted against corresponding values postpartum in such a way that the values of each investigation during pregnancy have been marked on the ordinate and those of the postpartum investigation on the abscissa. Thirteen blood sugar determinations were made during each glucose tolerance test, the first four fasting. The fasting values (1,2,3,4) during pregnancy are lower and fall below the identity line. (If they lay on this line it would indicate that the values were identical in pregnancy and postpartum.) The first two values after glucose ingestion (and the third at three months) are also below the identity line. The other values all are higher during pregnancy, except the last two (numbers 12, 13). Here again the seven-months' investigation is an exception as all values from No. 7 are higher than postpartum, i.e., above the identity line.

As shown in table 2 and figure 3, the initial value following intravenous injection of 50 ml. of glucose in 50 per cent solution is lower during than after pregnancy but recovery is slower during pregnancy and so the two curves cross.

The frequency of glycosuria following administration of glucose was as follows: 45 per cent in the third month, 57 per cent in the fifth month, 64 per cent in the seventh month, and 48 per cent in the ninth month. Glycosuria was found in 11 per cent postpartum.

TABLE 2

Intravenous glucose tolerance tests in eleven normal women. Mean blood sugar values during and after gestation (see also figure 3)

Minutes	During gestation	Postpartum
0	90.9	100.4
+2	289.5	330.9
+5	250.3	287.7
+10	209.4	244.2
+25	156.7	150.0
+40	124.2	108.7
+55	105.4	95.6
+70	94.5	92.0
+85	87.7	92.2
+100	84.4	94.9
+115	83.8	93.1

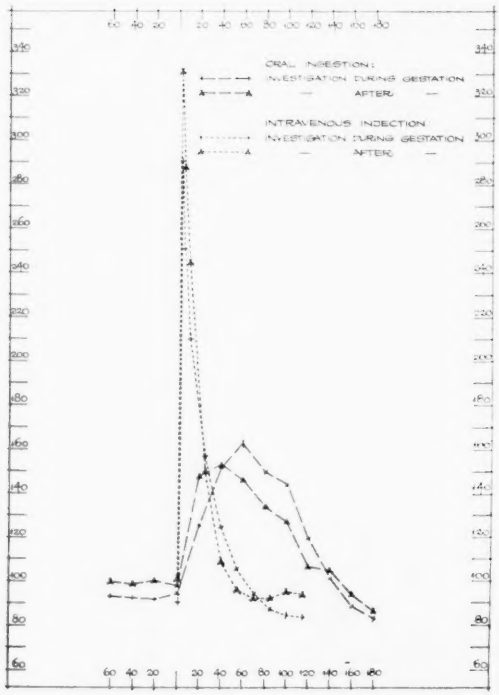

FIG. 3. Mean curves for eleven normal subjects. Intravenous and oral glucose tolerance curves during and after pregnancy. Blood sugar values in milligrams per 100 milliliters are shown on the ordinate; minutes before and after injection on the abscissa.

Fasting glycosuria was not found in any case. Glycosuria was thus most frequent in the seventh month.

Comment. The lower fasting blood sugar during pregnancy and the lower initial value following intravenous administration of glucose on the one hand and the higher increment and later peak in the oral curves (as well as the slower decrease in the oral and intravenous curves, on the other hand) might appear to represent opposite alterations of carbohydrate metabolism. The former might appear to represent intensification of blood sugar reduction and the latter a shift in the direction of a diabetic curve.

The intravenous curves give the impression that the lower initial value is simply a question of distribution, whereas the slower recovery is more suggestive of a true alteration in blood sugar regulation which would be present even though the initial value were not altered. In pregnant diabetics alterations in carbohydrate metabolism are represented by the increased tendency to insulin reactions in the second and third months and by the increased insulin requirement, especially in the seventh and eighth months.[11,12] In this connection it may be mentioned that, in patients with insulin-producing islet cell adenomas, spontaneous remission of the hypoglycemic attacks may occur during pregnancy.[13] Thus, the signs of augmented carbohydrate utilization must be considered secondary. It is concluded, therefore, that the effect of pregnancy upon the carbohydrate metabolism in normal subjects is a shift in the direction of diabetes, the shift being most pronounced at the beginning of the last trimester.

B. *Possible Prediabetics (Groups B 1 and B 2)*

Investigations similar to those described above were made in a group of forty-one women, each of whom had previously given birth to at least one child weighing more than 4,000 gm. Eleven such pregnant women were subjected to intravenous glucose tolerance tests.

Investigations on these forty-one subjects were conducted along the same lines as those on the twenty-eight normal pregnant subjects, but only thirteen mothers of large infants (group B 1) had four investigations during pregnancy, whereas the remainder (group B 2) were tested only once after the thirty-third week of pregnancy. All subjects were studied after delivery.

The women in group B 1 had previously given birth to a total of twenty-four infants with an average birth weight of 4,246 gm., and from the pregnancies under study all but one were delivered of live infants with an average birth weight of 3,846 gm. The sixty-four infants previously borne by the women in group B 2 had had an average weight of 4,097 gm., and the children they bore now weighed 3,852 gm. on the average. In seventeen of the forty-one cases labor was induced. The infant mortality in previous deliveries had been 13.6 per cent. The present pregnancy was terminated by the birth of living infants in forty. The mothers in group B ranged in age from twenty-one to thirty-four years and 29 per cent were more than 15 per cent overweight.

Tables 3 and 4 give the results of the tolerance tests in groups B 1 and 2, and figure 4 depicts the fasting blood sugar values and the glucose tolerance tests in group B 1. It is evident that the results correspond exactly to those in group A. This is confirmed by statistical analysis:

> In group B 1 the fasting blood sugar value was lowest in the third pregnancy investigation (at seven months) and it was significant at the 5 per cent limit. Postpartum it was higher in both groups than during pregnancy (0.1 per cent limit). In group B 2 the maximum increase was greater during than after pregnancy (0.1 per cent limit), and the peak occurred later (1 per cent limit). Corresponding find-

TABLE 3

Group B 1. Oral glucose tolerance tests in thirteen mothers of big infants (see also figure 4)

Minutes	Months of pregnancy 3	5	7	9	Postpartum
—60	94.4	93.2	89.2	95.3	106.0
—40	92.4	93.2	87.5	94.7	104.8
—20	90.8	92.3	88.4	95.7	103.4
0	91.4	91.8	88.0	95.6	104.4
+20	140.3	138.3	127.2	125.6	143.8
+40	163.7	162.2	154.6	156.4	169.4
+60	157.2	157.3	160.7	154.3	159.8
+80	139.6	144.1	152.2	144.9	140.0
+100	133.3	133.9	142.6	135.9	132.0
+120	119.6	119.5	128.6	120.4	122.2
+140	104.5	106.8	118.3	111.8	110.8
+160	94.4	101.5	111.3	102.7	100.1
+180	90.0	95.1	108.6	94.9	94.9

TABLE 4

Group B 2. Oral glucose tolerance tests in twenty-eight mothers of big infants. Only one investigation during pregnancy and one postpartum (see text)

Minutes	During gestation	Postpartum
—60	93.5	102.5
—40	93.3	102.1
—20	93.1	100.8
0	93.2	100.8
+20	130.6	146.5
+40	159.7	165.1
+60	166.4	160.8
+80	153.6	137.9
+100	143.8	129.2
+120	122.1	115.3
+140	110.1	106.8
+160	98.4	97.6
+180	90.7	96.7

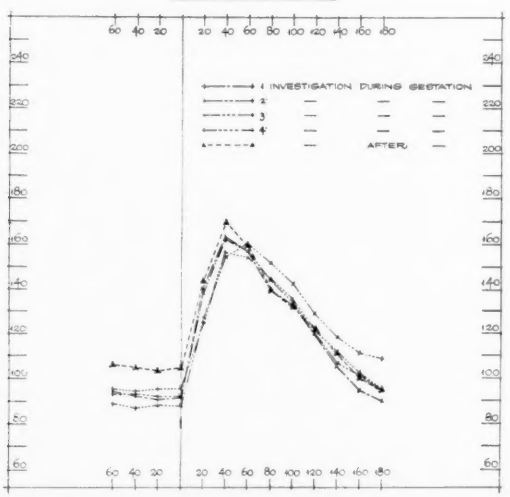

FIG. 4. Mean curves for thirteen pregnant women who had previously borne large infants. Blood sugar values in milligrams per 100 milliliters are shown on the ordinate; minutes before and after ingestion on the abscissa. Cf. figure 1.

ings were not definitely demonstrable in the B 1 group, but the trend was in the same direction. The blood sugar value two hours after administration of glucose was highest in the third pregnancy investigation in group B 1. In group B 2 this value was lower after than during pregnancy. This was also not definitely demonstrable in group B 1, but again the tendency was in the same direction as in group B 2.

Among mothers of large babies the blood sugar frequently exceeds 120 mg. per 100 ml. two and one-half hours after the administration of glucose. Thus, in the third (seven months) pregnancy investigation, seven out of thirteen in group B 1 exceeded that limit, and two in the fourth investigation. When investigated during pregnancy (corresponding approximately to the fourth investigation in groups A and B 1) the blood sugar of seven out of the twenty-eight patients in group B 2 exceeded 120 mg. per 100 ml. at two and one-half hours.

From table 5 it will be seen that the intravenous glucose tolerance curves were the same as in normal subjects, i.e., there was a less marked increase and a slower decrease in the level of blood sugar during pregnancy.

TABLE 5

Intravenous glucose tolerance tests in eleven mothers of big infants

Minutes	During gestation	Postpartum
0	92.7	104.8
+2	286.5	345.2
+5	246.3	313.3
+10	212.6	269.1
+25	162.3	158.2
+40	128.4	106.0
+55	109.0	92.4
+70	95.3	93.9
+85	87.0	97.5
+100	86.0	94.8
+115	88.4	98.0

The frequency of glycosuria varied approximately as in normal subjects. In the four stages of pregnancy it was 40 per cent, 38 per cent, 61 per cent, and 58 per cent. After delivery it was 17 per cent.

Comment. It is evident that the findings in women who had previously borne large infants are similar in many respects to the findings in normals. In order to evaluate the validity of calling the mothers of big infants possible prediabetics a detailed comparison of the findings must be made.

C. *Comparison of the findings in normals and in mothers of big infants*

Three findings stand out: (1) The tendency for the blood sugar to exceed limits of normal during pregnancy

is more outstanding among mothers of big infants, as mentioned above; (2) all values appear to be elevated in the mothers of large infants as compared with the normal subjects. The validity of this impression can be partially substantiated by the statistical findings:

Analysis showed that the fasting blood sugar values were higher in groups B than in group A and that this is significant in the ninth month. At other times the tendency is in the same direction. Furthermore, the maximum increase is greater in group B 2 than in group A, while no significance was found in this respect for group B 1, which, however, shows the same tendency. Finally, the time of the peak is later in group B 2 than in group A during pregnancy. Again, the tendency is the same for the entire B group, although significance could be shown only in one instance. As regards the blood sugar value at the end of two hours, results were not conclusive.

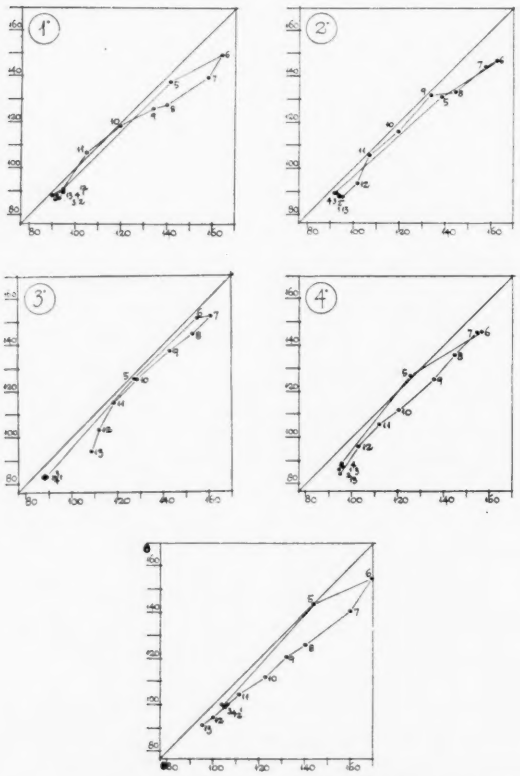

FIG. 5. Mean curves. Blood sugar values in milligrams per 100 milliliters at four investigations during pregnancy and one after delivery of twenty-eight normal women plotted against corresponding values of thirteen women who had previously given birth to large infants. Cf. text and figure 2. Note that practically all values are located below the identity line, i.e., the blood sugar values of mothers of big infants are higher than those of normal women. 1. at three months 2. five months 3. seven months 4. nine months 5. postpartum.

These findings are charted in figure 5 (cf. figure 2) in which the blood sugar values for normal subjects are plotted against the corresponding values for the mothers of large infants of group B 1. Clearly, the latter are located on their own side of the identity line, i.e., show higher values in practically all the investigations.

(3) The third conspicuous finding concerns the intravenous glucose tolerance tests. In the nonpregnant state the early increase in blood sugar is greater in groups B 1 and B 2 than in group A, but during pregnancy there is no difference. Since the dose of glucose was the same, the overweight mothers of large infants might be expected to show a less marked increase. The findings suggest that the blood sugar depressing factor of pregnancy is more active in mothers of large infants.

Comment. It must be concluded that the mothers of big infants exhibit higher blood sugar values than mothers of normal infants and also that the big babies during pregnancy receive a more copious flow of glucose via the maternal blood.

D. *An attempt to sort out the prediabetic patients*

The B groups were too small to permit subdivision into groups possessing different properties. Any attempt at subdivision meets with the difficulty which would also be encountered with a considerably larger series, viz. overlapping of important variables such as age, hereditary predisposition to diabetes, number of previous pregnancies, overweight in the pregnant and nonpregnant state, little weight loss after delivery, and the size of the infants, both in the previous and present pregnancy.

Some impression of the importance of the individual factors may be gained, however, and this impression proved a basis for further analysis. First, there seemed to be a tendency for a higher maximum and a slower decrease of the glucose tolerance curve with increasing age. This tendency is not very pronounced, and it is far from sufficient to explain the difference in the levels. Second, the glucose tolerance curves were different in pregnant women who were overweight postpartum and in those who had again given birth to large babies. The fasting blood sugar was relatively high, whence the curve rose abruptly to a maximum at the upper limit of normal and fell abruptly from the peak to values below the fasting level, after which it returned slowly to the initial value. These curves appear to correspond to the so-called "lag" or "oxyhyperglycemic"* curves.[14] (*See footnote page 443.*) They are not uncom-

mon in obese subjects and have been found previously in pregnancy.[15] They are interpreted as prediabetic curves by many authors.

There were nine women with oxyhyperglycemic curves, obesity and big infants in the observed pregnancies. These women possessed other common properties. First, there was a fairly high fertility rate with a total of twenty-five pregnancies. Two of these had ended in abortion (a low frequency) and four in stillbirths (a high frequency). The birth weights of the infants previously borne was high (4,347 gm.). Five infants had weighed more than 5,000 gm. Eight in the present series were delivered of living infants with an average birth weight of 3,861 gm., but the delivery was induced before term in five. The average body weight of the mothers was 96 kg., with a maximum of 128 kg.

Comment. It is probably justifiable to regard women with high fertility, particularly large infants with high perinatal mortality, and with marked obesity and "oxyhyperglycemic" glucose tolerance curves as potential diabetics.

SUMMARY

Determinations are presented of fasting blood sugar, oral and intravenous glucose tolerance tests, and glycosuria in pregnant women. The results are compared with similar investigations on the same persons in a nonpregnant condition. It was found that in pregnancy the fasting blood sugar was decreased, the oral glucose tolerance test was shifted in a diabetic direction, and the frequency of glycosuria was higher during pregnancy. These changes were most marked at seven months, at which stage the glucose tolerance curve was often in the abnormal range. Moreover, the blood sugar following intravenous injection of glucose did not rise as high during as after pregnancy, while the intravenous glucose tolerance curve was more prolonged during pregnancy. It is concluded that the most important alteration in carbohydrate metabolism in normal pregnancy is a shift in a diabetic direction, and thus, that normal pregnancy exerts a diabetogenic effect.

The same type of gestational blood sugar changes were found in normal pregnant women and in women who showed a tendency to bear big infants. However, the latter more often had abnormal glucose tolerance curves during pregnancy, and their blood sugar values on the whole were shifted toward a higher level. It seems possible to sort out among them a category with marked obesity, a history of high fertility and delivery of particularly big infants with a high perinatal mortality. Their oral glucose tolerance curves showed an "oxyhyperglycemic" course. They are considered potential diabetics.

*Such curves are characterized by the abrupt rise and fall and the high peak. This course of the curve may be looked upon as an exhaustion reaction of the blood sugar regulation.

SUMMARIO IN INTERLINGUA

Valores de Sucro Sanguinee Durante le Pregnantia in Gravidas Normal e Possibilemente Prediabetic

Es presentate le resultatos de determinationes del sucro sanguinee in stato jejun, de tests de tolerantia pro glucosa oral e intravenose, e de evalutationes del glycosuria in feminas pregnante. Iste resultatos es comparate con simile investigationes in le mesme subjectos in condition non-pregnante. Esseva trovate que in le pregnantia le nivellos del sucro sanguinee in stato jejun esseva reducite, le resultato del test de tolerantia pro glucosa oral esseva displaciate in le direction de diabete, e le incidentia de glycosuria esseva augmentate. Iste alterationes esseva le plus marcate post septe menses de graviditate. A iste tempore le curva de tolerantia pro glucosa esseva quasi in le region anormal. In plus, le nivello del sucro sanguinee post injectiones intravenose de glucosa non montava al mesme altor durante le pregnantia como post illo, e le curva de tolerantia pro glucosa intravenose esseva plus prolongate durante le pregnantia. Es concludite que le plus importante alteration del metabolismo de hydrato de carbon in pregnantia normal es un displaciamento in le direction de diabete e assi que pregnantias normal exerce un effecto diabetogenic.

Identic typos de alteration gestational del sucro sanguinee esseva trovate in gravidas normal e in gravidas tendente a parturir large infantes. Tamen, iste ultimas habeva plus frequentemente anormal curvas de tolerantia pro glucosa durante le pregnantia, e in illas—a generalmente parlar—le valores del sucro sanguinee se displaciava verso plus alte nivellos que in le gravidas normal. Il pare possibile differentiar inter illas un subcategoria con marcate grados de obesitate, un historia de alte fertilitate, e le tendentia de parturir exceptionalmente large infantes con alte mortalitate perinatal. Lor curva de tolerantia pro glucosa oral sequeva un curso "oxyhyperglycemic." Illas es considerate como diabeticas potential.

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On Teaching Diabetes

How Can the Practice of Medicine in the Treatment of Diabetes Be Improved?

The largest group of physicians who need training in improving diabetic care is practicing physicians. It was the consensus that a multiple and varied approach can be effective in the postgraduate education of practicing physicians. The first approach might be the same as that mentioned in regard to small hospitals. Through contact with an interested and informed internist who is available for consultation and for organization of subsidiary diabetic care and teaching, the practitioner can learn more about diabetes. In addition, postgraduate courses in diabetes such as those organized by the American Diabetes Association, are very helpful; but this particular program, valuable as it is, is limited to a relatively small number of physicians. Postgraduate courses in diabetes could be expanded to a great extent by medical schools and by local medical organizations at the state or regional level. In addition, teams from nearby medical centers might go out to local and regional meetings to stimulate interest in diabetes. This is being arranged in several states. Programs on diabetes could be incorporated in monthly staff meetings of community hospitals at which attendance is compulsory. Many practitioners could be reached through the Academy of General Practice. It is hoped that the least that could be accomplished through these approaches is to instill the attitude toward diabetes which we would like to see in physicians. Finally, it was suggested by some that teaching by mail with the help of visual aids be considered. Such concerted teaching programs by mail might be financed by interested agencies such as the American Diabetes Association, the United States Public Health Service, and possibly might be supported also by private organizations.

The second part of our discussion was concerned with possible improvements in the training and education of the patients. Two main questions can be asked: (1) How is the training best accomplished? and (2) What should the patient be taught? In regard to ways of teaching, it was the consensus that individual instruction by the physician as well as by the dietitian and nurse is still of great importance. In addition to individual attention, much can be gained by the patient's attending classes held by diabetes clinics in hospitals. The organization of classes for group therapy is one of the most important services which can be rendered for diabetic patients. Such classes may be conducted by physicians, dietitians and nurses.

In addition to these forms of therapy, there are several supplementary aids. Diabetic lay organizations can help in keeping up the patient's interest in self-education and should be supported by physicians, dietitians and nurses. Visiting public health nutritionists and nurses are available in many states to visit patients in outlying districts who cannot come to group meetings. Attempts should be made to stimulate and to encourage dietitians to go into the practice of diet therapy. There are many married dietitians who might be willing to work in such a capacity on a part-time basis and to whom physicians could send their patients for diet instruction. For diabetic children the availability of diabetes camps might be expanded.

By Stefan S. Fajans, M.D., in
Teaching and Research in Diabetes,
Charles C Thomas, Springfield,
Illinois, pp. 22-23, 1960.

Diabetes Mellitus and Pregnancy

Further Experience with Control of Perinatal Fetal Mortality

Antonio L. Court, M.D., Palmer H. Futcher, M.D., and W. Newton Long, M.D., Baltimore

The management of the diabetic patient during pregnancy continues to be a challenge and a subject of great interest, both to the obstetrician and the internist. Improved regulation of diabetes restored the regularity of ovulation and has expanded the number of young patients who live through the reproductive years. Thus, it is estimated that there are 80,000 diabetic women of childbearing age in the United States, and that 50,000 juvenile diabetic subjects are or soon will be potentially childbearing.[1] According to the studies of the Chicago Lying-In Hospital and the Boston Lying-In Hospital, the incidence of deliveries of diabetic patients in large maternity hospitals is one in 1,000.[2]

Medical institutions around the world continue to report their individual statistics. The data from The Johns Hopkins Hospital on pregnancies in diabetic subjects observed during the eleven-year period from 1942 through 1952 have been reported;[3] included was information on fifty-six pregnancies handled by the same technics utilized in this study. Presented below are data on pregnancies in diabetic subjects occurring during the subsequent six-year period.

MATERIAL AND METHODS

From Jan. 1, 1953, to Dec. 31, 1958, there were ninety-seven deliveries in eighty-five diabetic patients at The Johns Hopkins Hospital. Of these, forty deliveries occurred in white patients and fifty-seven in nonwhite patients. This ratio is similar to that of the previous report. The group included patients of both clinic and private status, the latter group comprising a small minority. Most of the clinic patients had been followed at the hospital's prenatal diabetes clinic during the last two trimesters of their pregnancy. A few had been followed at county clinics prior to their hospital admission for delivery. There were others who came to the hospital for delivery without prenatal care, and others still who came to the prenatal clinic very late in pregnancy.

As in the previous report,[3] only cases in which the diagnosis of diabetes was certain were included in the series. The criterion for the diagnosis of diabetes was the finding before or during the pregnancy of two or more fasting blood sugar levels above 130 mg. per 100 ml. as measured by the Folin-Wu technic[4] or in excess of 115 mg. per 100 ml. as measured by the glucose oxidase method.[5] A glucose tolerance test, employing 100 gm. of glucose, was performed on subjects suspected of manifesting a very mild form of diabetes; the results were interpreted by standard criteria.[6]

The eight pregnancies in private patients were supervised by members of the private visiting staff—an internist, and an obstetrician; the latter conducted the delivery. Clinic patients were delivered by members of the hospital house staff. Clinic patients were followed at the prenatal diabetes clinic, supervised by an obstetrician (WNL) and an internist (PHF). There the patients were seen at intervals of one to two weeks. Certain patients were seen in consultation by an ophthalmologist for an evaluation of possible progression of vascular disease. Measurement of fasting blood glucose and urinalysis were performed at the time of the return visits. During the intervals, the patients examined their urine for sugar before each meal.

An effort was made to regulate the diabetes as strictly as possible, to maintain the fasting blood sugar below 130 mg. per 100 ml. as measured by the Folin-Wu method and to avoid reactions or great swings in blood sugar levels throughout the day. A diet was prescribed offering each day 150 to 250 gm. of carbohydrate, 100 gm. of protein, and the balance of calories as fat, usually in amounts of forty to ninety grams. Effort was made to reduce the weight of obese women during their pregnancies. Crystalline, Protamine-Zinc, NPH and, more recently, Lente insulin were employed. An occasional patient needed no insulin. None of the oral hypoglycemic compounds was used. Estrogens and progesterone-like drugs were not employed.

The majority of patients were admitted to the hospital for regulation of their diabetes over a short period

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at some stage of their pregnancy. At the thirty-seventh week of gestation, or before if indicated, the appropriate type of delivery and the possible need for early delivery were reviewed. If the diabetes mellitus was mild, the pregnancy uncomplicated, and the fetus estimated to weigh between 3,000 and 3,400 gm., the patient was allowed to deliver vaginally. In this group of mildly diabetic subjects, if the uterine cervix was favorable, labor was induced at the end of the thirty-seventh week, by stripping and rupturing the membranes. If the condition of the uterine cervix was unfavorable for induction, the patient was hospitalized until such a procedure became feasible, or until a spontaneous onset of labor ensued.

If, however, the pregnancy was complicated by toxemia, progressive retinopathy or other vascular abnormality; if the duration of the diabetes was more than fifteen years, or juvenile in origin, or if the fetus was estimated to be large, elective cesarean section was carried out at the end of the thirty-seventh week, or sooner in the presence of serious complications. Previous infant loss or other obstetric factors were also regarded as indicating the desirability of abdominal delivery. These rules were not rigidly adhered to but were generally followed.

As in the past, our pediatric consultants have treated the infants of diabetic mothers as if they were premature, regardless of birth weight.[8]

RESULTS

In reporting our observation, we have employed the nomenclature recommended by the World Health Organization. Thus a "viable" fetus or infant is one weighing over 400 gm. or one delivered at the twentieth week of gestation, or after. The delivery of a dead fetus falling into this category is classified as a "stillbirth."

During the six-year period covered in the study there were 18,972 deliveries of all mothers at The Johns Hopkins Hospital, giving an observed incidence of deliveries of diabetic patients of 0.51 per cent or five in a thousand. This incidence is higher than that reported in other large maternity hospitals.[2] As shown in table 1, ninety-five out of the ninety-seven infants delivered at the hospital weighed 1,000 gm. or more. Of these, seventy-nine survived. A perinatal mortality rate of 18.6 per cent was observed. This total was partially accounted for by twelve stillbirths, in ten of which the birth weight was over 1,000 gm. In addition, there were six neonatal deaths. The incidence of perinatal infant death was essentially the same in the white and Negro racial groups, being 20.0 and 17.5 per cent. This mortality rate of 18.6 per cent is identical with that observed in the infants produced in fifty-six pregnancies in which the medical and obstetrical management was similar.[3]

TABLE 1

General data on deliveries of diabetic mothers at The Johns Hopkins Hospital, Jan. 1, 1953, through Dec. 31, 1958

	Number	Per cent
Women delivered	85	
Deliveries	97	
Infants 1,000 gm. and over	95	
Perinatal mortality, total	18	18.6
Perinatal mortality, over 1,000 gm.	16	16.8
All stillbirths	12	
Stillbirths over 1,000 gm.	10	
Neonatal deaths	6	
Surviving infants	79	
Twins	0	
Maternal deaths	0	

Data regarding toxemia are presented in tables 2, 3 and 4. A patient's disorder was classified as preeclampsia or hypertensive disease according to criteria set by the American Committee on Maternal Welfare.[7] Preeclampsia, according to this classification, indicates the development after the twenty-fourth week of pregnancy of one or more of the following manifestations: a sys-

TABLE 2

Incidence of toxemia in ninety-seven pregnancies

	Pregnancies	
	Number	Per cent
Preeclampsia	17	17.5
Hypertension	22	22.7
Toxemia total	39	40.2

TABLE 3

Perinatal mortality and type of toxemia

	Pregnancies	Deaths	
	Number	Number	Per cent
No toxemia	58	10	17.2
Preeclampsia	17	2	11.8
Hypertension	22	6	27.2

TABLE 4

Toxemia and perinatal mortality correlated with White's classification of diabetes

	Total Pregnancies		Toxemic Pregnancies		Deaths	
Class	Number	Per cent	Number	Per cent	Number	Per cent
A	5	5.2	1	20.0	0	0.0
B	54	55.7	20	37.1	9	16.6
C	21	21.7	7	33.3	4	19.0
D	14	14.4	9	64.3	3	21.4
E	1	1.0	0	0.0	1	100.0
F	2	2.1	2	100.0	1	50.0

tolic blood pressure of 140 mm. of mercury or more or a rise of 30 mm. or more above the usual level; a diastolic pressure of 90 mm. or more, or a rise of 15 mm. or more above the usual level; proteinuria of significant degree; or persistent edema of the hands or face. Hypertensive vascular disease was diagnosed in those women manifesting a systolic blood pressure of 140 mm. or more or a diastolic pressure of 90 mm. or more before the twenty-fourth week of gestation, usually persisting after delivery, unrelated to pregnancy, and demonstrated to be present in the nonpregnant state.

Preeclampsia or hypertensive disease was present in 40.2 per cent of the pregnancies. Hypertensive disease comprised more than half of the group; it was present in 22.7 per cent of the ninety-seven pregnancies. Perinatal loss was highest in this group with hypertension, being 27.2 per cent, compared with the rate of 11.8 per cent in the preeclamptic group. This finding suggests that hypertensive vascular disease is a very serious complication in these diabetic patients. The fact that preeclampsia occurs predominantly in relatively younger women[8] in whom diabetes may have been present for a shorter period of time may contribute to its less frequent association with perinatal death. We cannot offer impressive evidence for this explanation, however, since in the present study there were fifteen patients with preeclampsia in one or two pregnancies whose mean age was 29.2 years whereas the mean age of twenty-two women with hypertensive disease was 31.9 years. It is interesting to note in table 3 that, whereas the perinatal loss in the pregnancies in the nontoxemic group was 17.2 per cent and closely approximated the over-all loss of 18.6 per cent, the perinatal loss in pregnancies complicated by preeclampsia was only 11.8 per cent. It must be recognized that some of these deviations may be due to chance, since the number of subjects in each group is small.

White's criteria[9] for classification of the severity of diabetes were used in the analysis of our data presented in table 4. White's classification is as follows: Class A—Glucose tolerance test slightly abnormal, no insulin needed, little dietary regulation; Class B—Onset of diabetes after twenty years of age or diabetes of less than ten years' duration, cases free of vascular disease; Class C—duration of diabetes long, ten to nineteen years, onset of diabetes between ages ten to nineteen, minimal vascular disease, such as retinal arteriosclerosis or calcification of the vessels of the legs alone; Class D—prolonged duration of diabetes for twenty years or more, onset of diabetes before age ten, evidence of vascular disease such as retinopathy, transitory albuminuria, or transitory hypertension; Class E—pelvic arteries calcified; and Class F—all patients with nephritis.

As can be seen from table 4, most cases fell in Classes B, C, and D, which collectively account for 91.8 per cent of the series. The majority (55.7 per cent) of the patients were classified as Class B, and manifested relatively few complications of diabetes. The infant mortality rate and the incidence of toxemia in all classes save A were elevated above the over-all average for the obstetrical clinic. No deaths occurred in the Class A group (five deliveries).

Of the fifty cesarean sections performed, in twenty-eight instances the patient had undergone a previous section (table 5). This is in sharp contrast to the finding in our previous study[3] that only three out of forty-four sections were not primary.

It will be noted that in table 6 consideration has been limited to those cases in which a living fetus was present at thirty-seven weeks of gestation. In this category there was observed a practically identical incidence of fetal death whether the infant was delivered by section or by vagina—8.3 and 10.3 per cent respectively.

Viable infant losses are reviewed in table 7. There were nine deaths in utero before labor (50 per cent of the group), three cases of death in utero during labor and six neonatal deaths. It is of interest that of the nine infants dying before labor, seven were the infants of mothers in whom control of diabetes was poor. For statistical purposes, we consider these seven deaths preventable. Of the remaining deaths, one was associated with hypertensive vascular disease and another occurred after the mother developed mumps at twenty-eight weeks.

Of the fetal deaths during labor, one involved a very

TABLE 5

Perinatal mortality related to method of delivery

Method	Deliveries		Deaths	
	Number	Per cent	Number	Per cent
Cesarean section	50	51.5	6	12.0
Vaginal delivery	47	48.5	12	25.5

TABLE 6

Perinatal mortality in eighty-seven pregnancies in which fetal heart was heard at thirty-seven weeks of gestation related to method of delivery

Method	Deliveries		Deaths	
	Number	Per cent	Number	Per cent
Cesarean section	48	55.2	4	8.3
Vaginal delivery	39	44.8	4	10.3

TABLE 7

Viable infant losses

Patient	Date fetal heart ceased	Infant weight	White class	Known duration diabetes	Maternal weight	Maximum dose insulin	Remarks*
	Week	Grams		Years	Pounds	Units/day	
		A. Fetal death in utero before labor					
1	36	3,220	D	4	138	30	Hypertension
2	30	2,420	B	1	242	60	Poor regulation, hypertension
3	27	645	E	11	183	15	Poor regulation
4	33	2,350	B	1	152	150	Poor regulation
5	33	1,845	F	17	129	74	Poor regulation, hypertension
6	33	1,725	D	11	172	60	Poor regulation
7	37	3,650	B	0	266	0	No regulation (diagnosed postpartum), preeclampsia
8	32	630	C	12	147	50	Mumps at twenty-eight weeks, preeclampsia
9	37	1,550	B	4	193	0	Poor regulation
		B. Fetal death in utero during labor					
10	39	4,250	B	1/12	187	0	Shoulder dystocia, poor regulation
11	39	5,600	B	2/12	300	15	Section, hysterectomy, hypertension, poor regulation
12	41	3,800	B	1/12	353	25	Extreme maternal obesity
		C. Neonatal deaths					
13	33	2,410	D	12	150	80	Section, hypertension, diabetic retinopathy
14	39	1,700	B	3	264	30	Section, duration of pregnancy questionable, hypertension
15	31	1,920	C	13	170	0	Microcephaly
16	33	2,980	C	11	130	90	Section
17	37	2,945	C	11	147	60	Section, multiple fetal anomalies, poor regulation
18	37	3,930	B	2	215	20	Section, fetal renal arteries thrombosed

*Unless delivery by cesarean section ("section") is indicated, deliveries were vaginal.

TABLE 8

Perinatal mortality and infant weight

Weight	Infants	Deaths	
Grams	Number	Number	Per cent
400-999	2	2	100.0
1,000-1,499	2	0	0.0
1,500-1,999	9	5	56.0
2,000-2,499	4	3	75.0
2,500-2,999	14	2	14.3
3,000-3,499	18	1	5.5
3,500-3,999	24	3	12.5
4,000-4,499	14	1	7.1
4,500-4,999	8	0	0.0
5,000-5,499	1	0	0.0
5,500 +	1	1	100.0

obese woman whose infant was injured during delivery; there was a question whether or not the fetal heart was heard on admission. A cesarean section and hysterectomy were performed. In another delivery complicated by shoulder dystocia, attempts at delivery before admission to the hospital had fatally mutilated the infant. The third instance involved the infant of a tremendously obese woman who presented in labor after a forty-one-week gestation period. The second stage of her labor was very prolonged.

Of six neonatal deaths, five occurred in infants who had been delivered by cesarean section. This group included the two infants manifesting anatomical anomalies. There was a third infant with thrombosed renal arteries. Another neonatal death occurred after a mother was delivered at thirty-three weeks by cesarean section because of hypertensive vascular disease and diabetic retinopathy. Hyaline membrane disease was found at autopsy in the lungs of the other two infants. It is to be noted that in no infant which was delivered vaginally was a hyaline membrane found at autopsy, although one infant who survived was treated for this condition for several days because the diagnosis was suspected.

Infant mortality is correlated with birth weight in

table 8. The lowest mortality rate, 5.5 per cent, occurred in the group weighing 3,000 to 3,499 gm. with one death in eighteen deliveries. Closely similar to this rate were those of the groups weighing between 2,500 and 2,999 gm. and between 3,500 and 3,999 gm., 14.3 and 12.5 per cent respectively. Death occurred more frequently in infants weighing below 2,500 gm.; there were ten deaths in seventeen deliveries, a 59 per cent mortality rate.

DISCUSSION

Most of the mothers we are reporting upon were observed in the prenatal diabetes clinic, where patients are seen on a referral basis. No special efforts have been made to find "prediabetic" subjects or women with Class A diabetes among the patients presenting themselves to the general obstetrical clinic through the routine use of glucose tolerance tests. Wilkerson's studies,[10] as yet incomplete, may demonstrate the desirability of such a procedure. We have performed glucose tolerance tests only in those patients in whom we suspected diabetes because of previous fetal losses, a family history of diabetes, previous large babies, or the presence or history of hydramnios, toxemia, or glycosuria. Therefore, the number of Class A diabetic patients in this series is less than that of other series now being reported.

Patients who were not followed in the prenatal diabetes clinic are included in the statistics. Seven fetal deaths occurred in utero in the infants of patients whose diabetes was poorly controlled (table 7). These patients represented some of those who either received no prenatal care, or those in whom habitual failure to follow instructions prevented reduction of hyperglycemia. These seven deaths are therefore considered preventable. When we consider this group in conjunction with the three deaths occurring during labor, we find that ten, or 55 per cent, of the deaths, were possibly preventable.

A problem found among patients who were followed in the clinic was inability or unwillingness of many patients to undergo hospitalization as often as was deemed necessary. This factor operated adversely, but the extent of its influence could not be estimated fully. Preeclamptic patients were admitted sooner and were treated more vigorously than those with hypertension. Their management included more sedation and earlier delivery.

Regarding the use of cesarean section, no hard and fast rules were followed. Each case was evaluated individually, consideration being given to all the variables involved. Inspection of tables 6 and 7 may lead to the conclusion that cesarean section was of no help or was even detrimental in some cases. It must not be forgotten, however, that this operation was often performed under the circumstances of increased incidence of maternal vascular disease. Further, sections were carried out in many instances in patients whose complications, such as fetal-pelvic disproportion, were obstetrical, although produced by diabetes. Cesarean section continues to be of value in the presence of toxemia and other situations in which early termination of pregnancy is indicated.

The perinatal mortality rate of 18.6 per cent observed in these ninety-seven recent pregnancies is identical with the 18 per cent recorded by us for an earlier series of fifty-six diabetic pregnancies which were conducted in a similar manner.[3] The only differences in the management of the two groups were that (a) relatively more patients were followed by local physicians in county clinics in the more recent period, (b) more emphasis was placed on restriction of the fat in the diet in the second series, and (c) more intermediate insulin (NPH or Lente) and less Protamine Zinc Insulin were used in the present series.

Various provocative reports on the outcome of diabetic pregnancies have been published describing considerable variations in fetal mortality.[11–16] These differences seem to depend on such factors as the number of Class A diabetic patients, the proportion of those with juvenile diabetes, the number of patients with inadequate prenatal care, the use of delivery employing induction and cesarean section and the number of those pregnancies excluded as "previable." Thus it often seems inappropriate to compare one series with another. The method of selection of the diabetic mother reported upon by Hagbard[13] was relatively similar to that we utilized, as were the clinical methods employed; differences included Hagbard's more frequent utilization of cesarean section at thirty-seven weeks of pregnancy and the physical dispersion of his observing clinics. Hagbard reported a total perinatal mortality of 26 per cent, with 14 per cent stillbirths and 12 per cent neonatal mortality.

It is clear that, given our incomplete knowledge of the cause of intrauterine death in diabetes, the rather simple educational background of a majority of our clinic population, and the late stage of pregnancy at which some of the subjects presented themselves for care, the technics we have employed do not prevent infant disaster in one of five pregnancies. This fetal wastage is approximately five times that recorded for all pregnancies at our hospital; it is, however, only half the fetal wastage (36 per cent) observed in our Obstetrical Clinic before the institution of a special service for diabetic mothers in 1948.[3] Our same technics employed in the care of an educationally and economically

more privileged group could be expected to effect a reduction in fetal loss below 18 per cent on the assumption that more satisfactory regulation of diabetes, and hence fewer fetal deaths, will ensue in this setting.

In this regard, the tabulated data available to us do not permit an exact comparison of the relative excellence of control of the diabetes in the mothers producing infants which survived with the degree of control achieved in the mothers whose fetuses did not survive. A review of the information on our series which is at hand suggests a higher incidence of impressive hyperglycemia in the mothers in whom the pregnancy was unsuccessful. Thus, in ten out of eighteen mothers encountering an unsuccessful pregnancy the control of diabetes was grossly unsatisfactory.

During the course of our study, our interest was directed toward the subject of neonatal infant hypoglycemia. The incidence and interpretation of this phenomenon are widely debated by those concerned with the care of the infants of diabetic women. Many observers have denied its occurrence except as produced by mismanagement of insulin administration on the day of delivery. In this series of ninety-seven deliveries, the blood sugar of many of the infants was measured. Neonatal hypoglycemia with serum glucose less than 25 mg. per 100 ml. occurred in ten infants. A common factor was sought for but not found. Maternal insulin dosage varied from zero to 115 units. No patient had preeclampsia; one had essential hypertension. Nine of the ten patients were delivered by cesarean section while one delivered spontaneously. The diabetes of all patients was classified as Class B or C. We do not attempt to explain the fact that at least 10.3 per cent of our infants manifested this difficulty, but we do feel that insulin dosage and toxemia were not consistently significant factors.

SUMMARY

Ninety-seven viable pregnancies in eighty-five women manifesting diabetes mellitus were observed at The Johns Hopkins Hospital between Jan. 1, 1953, and Dec. 31, 1958. The observed incidence of diabetic deliveries during that period was five in 1,000 deliveries.

Measures employed in the management of the pregnancies included strict regulation of the diabetes; delivery when feasible at the end of the thirty-seventh week, by cesarean section in selected cases; and treatment of the infant as if he were born prematurely.

There were eighteen perinatal deaths, equivalent to an infant mortality rate of 18.6 per cent. This is identical with the rate we reported previously for a similarly treated series of fifty-six diabetic pregnancies.[2] Seven of the intrauterine deaths in the present series occurred in the infants of mothers whose diabetes was uncontrolled; another three deaths occurred during labor. These ten deaths, half of the total of eighteen, are considered preventable. No maternal deaths occurred.

It is felt that more frequent hospitalizations, sometimes for prolonged periods of time, would be of benefit in our particular clinic population.

In our small series long-standing hypertension was even more dangerous to the fetus than was preeclampsia.

Ten instances of neonatal fetal hypoglycemia, with blood sugars below 25 mg. per 100 ml., were observed in association with the deliveries. No suitable single explanation was found.

SUMMARIO IN INTERLINGUA

Diabete Mellite e Pregnantia. Nove Experientias in le Combattimento de Perinatal Mortalitate Fetal

Novanta-septe viabile pregnantias in octanta-cinque feminas con manifeste diabete mellite esseva observate al Hospital Johns Hopkins inter le 1 de januario 1953 e le 31 de decembre 1958. Le incidentia de parturitiones diabetic observate durante ille periodo esseva cinque in 1000.

Le mesuras empleate in le manipulation del pregnantias includeva un stricte regulation del diabete, effectuation—in tanto que possibile—del parturition al fin de trenta-septe septimanas de gestation (per section cesaree in seligite casos), e tractamento del infante como si ille esseva nato prematur.

Esseva contate dece-octo mortes perinatal, equivalente a un mortalitate infantil de 18,6 pro cento. Isto es identic con le incidentia previemente reportate per nos pro un similemente tractate serie de cinquanta-sex pregnantias diabetic. Septe del mortes intrauterin in le presente serie occurreva in le infantes de matres in qui le diabete non esseva stabilisate. Tres mortes additional occurreva durante le labores. Iste mortes—representante plus que un medietate del total de 18 mortes—es considerate como prevenibile. Nulle morte materne occurreva in le serie.

Es opinate que plus frequente hospitalisationes—in certe casos pro prolongate periodos de tempore—esserea de beneficio in nostre typo de population clinic.

In le presente serie—que non esseva extense—hypertension de longe durantia esseva mesmo plus periculose pro le feto que preeclampsia.

Esseva observate dece casos de neonatal hypoglycemia fetal, con nivellos de sucro sanguinee de infra 25 mg per 100 ml al tempore del parturition. Nulle satisfacente explication de iste constatation esseva trovate.

ACKNOWLEDGMENT

Miss Ella Rowe, R.N., of the obstetrical outpatient nursing staff, rendered valuable assistance in the management of the patients reported upon in this study. Dr. Court held a Department of Medicine fellowship in the Private Patient Clinic during part of the period devoted to this study. Dr. Thaddeus E. Prout of the Department of Medicine reviewed an early stage of the manuscript.

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On Teaching Diabetes

Laboratory Work on Diabetes in the Basic Science Departments

A group of medical students may be given some hypophysectomized rats to follow for several weeks. The curves of these rats are compared with those of: (1) control rats subjected to sham operations and (2) hypophysectomized rats treated with growth hormone. After these animals have been observed for three weeks, an appropriate dose of insulin is injected in each. Then the student can see the marked hypersensitivity of hypophysectomized rats to insulin and can compare this reaction to that of normal animals and of animals treated with growth hormone. After these studies are completed, the animals can be killed and the histologic width of epiphyseal cartilage of each can be measured. In this way the respective effects on cartilage growth produced by hypophysectomy and by growth hormone can be estimated. Then, simply by weighing the other endocrine glands, the medical student can readily demonstrate that hypophysectomy produces marked atrophy of the adrenal, thyroid, and gonads, and that the weights of these glands are not restored by administering growth hormone. Similar experiments can be devised to show graphically the effects of the adrenotropic and gonadotropic hormones.

These and many similar experiments can be performed within the first year of the medical school curriculum. Since they are somewhat more complex than the traditional experiments, the students must plan for them with special care. If, for example, they are to collect twenty-four-hour urine excretions over a two- to three-week period (including Saturdays and Sundays) and if they are to administer insulin at specified intervals, they must rearrange their schedules accordingly.

Setting up a group of experiments of the type outlined does not necessarily require an integrated teaching program. Even with a classical departmentalized approach one can use a portion of the laboratory time ordinarily allotted to teaching anatomy, physiology, biochemistry and set up a group of endocrine experiments toward the end of the freshman year of medical school. Since each student would be involved in one of a possible dozen endocrine experiments, it is desirable to have each group present the results of its particular experiment to the entire class, to discuss the scientific background of the experiment, and to answer the questions of their fellow students.

By Arnold Lazarow, M.D., in
Teaching and Research in Diabetes,
Charles C Thomas, Springfield,
Illinois, pp. 45-46, 1960.

Observations on the Microaneurysms of Diabetic Retinopathy

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The occurrence of microaneurysms in the diabetic retina was reported by MacKenzie and Nettleship in 1879,[1] but in subsequent clinical publications (Hirschberg[2]) this observation was disregarded and the structures were described as punctate hemorrhages. Therefore, the significance of capillary aneurysms in diabetic retinopathy was not realized until 1943, when they were rediscovered by Ballantyne and Loewenstein.[3] Although these aneurysms have been studied extensively in both histological sections (Ballantyne et al.[4-6]) and flat preparations (Friedenwald,[7,8] Ashton[9]), much of their basic etiology has remained obscure. This study was undertaken in the hope that added knowledge might be gained by using technics (Hausler and Sibay[10,11]) which allow observation of both the general outlines and the details of the retinal blood vessels in flat preparations.

METHODS AND MATERIALS

Eyes from diabetic patients were supplied by the Eye Bank of Canada (Ontario Division), after the corneas had been used for grafting. The eyes had been kept in glass jars (forming a moist chamber) at +4°C. for periods varying from a few hours to several days. They were then injected through the central retinal vessels, as outlined previously, and the retinas viewed in cleared, flat preparation (Hausler and Sibay[11]). In this method a silver salt solution on reduction outlines the boundaries of the endothelial cells. After partial penetration of the endothelium, the solution stains the neurofibrils, reticular fibers and the perimysium of smooth muscle cells surrounding the arteriolar walls.

OBSERVATIONS

When viewed under low magnification (figure 1 and figure 2) our preparations are confirmatory of previous studies on the morphology of the microaneurysms. Their diameters range from 25 to 100 μ; their shapes vary, the majority appearing as saccular pouchings from one side of a capillary wall, leaving the other side unaffected. Examples of pouching affecting the capillary wall in its whole circumference can be found. Some of the aneurysms lie at kinks in the capillary loops, as described by Ashton.[12] Others are on straight sections of capillaries. Frequently, an aggregation of aneurysms surrounds an avascular area. The majority of the aneurysms lie in the outer capillary plexus towards the venous side of the capillary loops, but it is not uncommon to see them in the inner plexus and close to arterioles.

FIG. 1. Diabetic retinopathy. Cleared retinal flat preparation, silver injected. Groups of microaneurysms surround avascular areas. All these aneurysms involve only one side of the capillary wall. X 40.

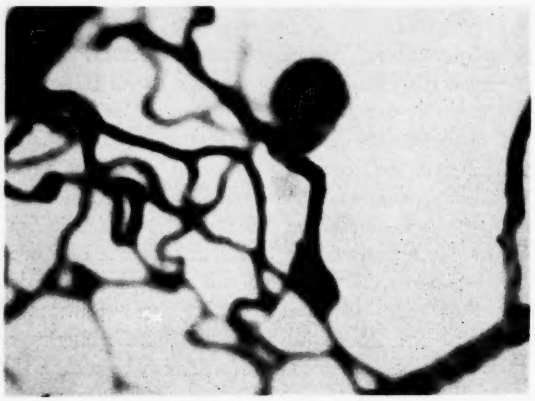

FIG. 2. Diabetic retinopathy. Cleared retinal flat preparation, silver injected. Typical microaneurysm arising from a capillary loop. X 400.

From the Departments of Physiology and Ophthalmology, Faculty of Medicine, University of Toronto, Toronto, Canada.

Under high magnification (figures 3, 4 and 5) some structural details are of interest. Where the pouching involves only one side of the capillary wall, the endothelial cells of the unaffected portion are of normal size and shape and have intact boundaries. The endothelial cells lining the sac are swollen, irregular and have interrupted boundaries, or are broken or missing. These cellular changes are commonly associated with thickening of the basement membrane, which increases to fifty to sixty times its normal width. Doubly refracting crystals may be present in the thickened membrane (figure 6).

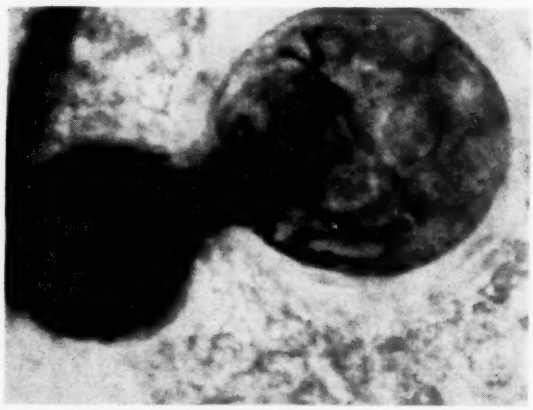

FIG. 3. Microaneurysm from diabetic retinopathy, silver injected. This aneurysm is lined by grossly swollen endothelial cells with irregular and partly disrupted boundary lines. The basement membrane is greatly thickened. X 1,850.

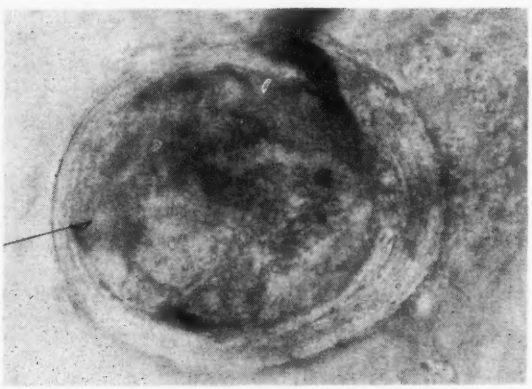

FIG. 4. Microaneurysm from diabetic retinopathy, cleared retinal flat preparation, silver injected. This aneurysm is lined only partially by swollen and disrupted endothelial cells. In one area (arrow) the endothelium is missing. The pouch is surrounded by a greatly thickened basement membrane. X 2,100.

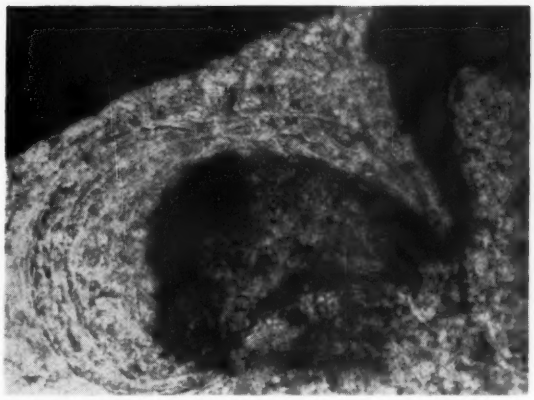

FIG. 5. Microaneurysm from diabetic retinopathy, cleared flat preparation, silver injected. This microaneurysm is lined with the amorphous residue of endothelial cells; intercellular boundary lines are not visible. A thickened basement membrane encases the devitalized area. X 1,850.

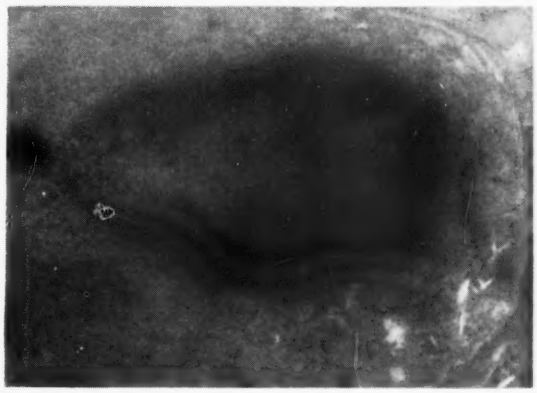

FIG. 6. Microaneurysm from diabetic retinopathy, cleared flat preparation. This aneurysm is encased in a thickened basement membrane with many crystals incorporated in its layers. X 2,000.

DISCUSSION

The aneurysms of diabetic retinopathy arise from capillaries. Capillaries have been considered to be formed of endothelial cells on the inner surface of a basement membrane, with surrounding pericytes lying at intervals along the vessel. It was held that the pericytes were situated outside the basement membrane, but Benninghof[13] and Löschke and Löschke[14] postulated that these cells were surrounded by the membrane. Recent electron microscopic studies indicate that the basement membrane is a thickened, external, double-lined wall of the endothelial cell (Kisch[15,16]), measuring about 300

Å across and having a fairly straight course. The internal cell membrane is thinner, measuring only about 80 Å, and has a highly irregular, scalloped outline. According to Monné[17] these membranes are thought to consist of alternating lamellae of protein and lipoid molecules.

Friedenwald,[7,8] using the PAS staining method, first described the thickened, intensely staining walls of the aneurysms. Our preparations show that these walls consist of enormously thickened basement membrane. If the basement membrane is formed by, or is an integral part of, the endothelial cells, the changes in the membrane may well be secondary to pathology in the endothelial cells themselves. A plausible sequence of events could be the following:

Localized degenerative changes in a patch of endothelial cells lead to swelling and abnormal deposition of catabolites in the adjacent basement membrane. The basement membrane becomes pouched out and swells to sixty times its normal thickness. It now shows the characteristic affinity for the PAS and the Lipoid Feyerter stains. The endothelial cells then are partially destroyed; finally they can no longer be demonstrated lining the pouch of the aneurysm. Once this has happened the aneurysm remains stationary or is obliterated by a microthrombus.

One might speculate that in diabetes the demand of the actively metabolizing retina on the supplying capillaries may become excessive and that this may result in localized endothelial damage. If the damage is mild, the affected cells produce thickening of the basement membrane. If the damage is severe and sudden, the cells of an affected area will be destroyed. The result will be a leaking blood vessel, producing a retinal hemorrhage.

SUMMARY

Studies of the microaneurysms of diabetic retinopathy in retinal flat preparations reveal endothelial changes in the pouched out areas of the capillary wall. This change is associated with an increase in the thickness of the adjacent basement membrane which, on occasion, swells to sixty times its normal width. It is suggested that the formation of microaneurysms has its origin in metabolically disturbed endothelial cells and, therefore, is an active process rather than a passive dilation of a weakened capillary wall.

SUMMARIO IN INTERLINGUA

Le Microaneurysmas de Retinopathia Diabetic

Studios del microaneurysmas de retinopathia diabetic in plan preparatos retinal revelava alterationes endothelial in le extrundite areas saccular del pariete capillari. Iste phenomeno es associate con un augmento del spissitate del adjacente membrana basilar le qual—in certe casos—attinge un largor de sexanta vices le dimension normal. Es proponite le these que le formation de iste microaneurysmas prende su origines in metabolicamente disturbate cellulas endothelial e es—per consequente—un processo active plus tosto que un dilatation passive del debilificate pariete capillari.

ACKNOWLEDGMENT

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Episodic Hyperkalemic Paralysis

Report of a Case of Adynamia Episodica Hereditaria Associated with Diabetes Mellitus

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The hereditary syndrome of recurrent paralysis of the skeletal musculature was first recognized as a clinical entity by Westphal in 1885.[1] In 1934, Biemond and Daniels[2] discovered low serum potassium values during these attacks of "familial periodic paralysis." Most cases were similar; however, some investigations revealed no change in serum potassium concentration during attacks and, in some instances, the administration of potassium was not beneficial.[3,4]

In 1952, Gamstorp and Mjönes began the investigation of a family afflicted with episodic paralysis and were impressed by certain features which differed from the established syndrome of familial periodic paralysis (FPP). Attacks were first manifest at a younger age, were usually of shorter duration and occurred more frequently. Sexes were equally affected as opposed to the usual male predilection. Episodes of weakness also occurred more commonly during the day and most frequently when at rest following exertion. A striking difference from FPP was noted when potassium metabolism was studied. During attacks the excretion was unchanged but serum potassium levels were increased. Also, the administration of small amounts of potassium precipitated paralysis. Gamstorp reported her studies in 1956, describing adynamia episodica hereditaria (AEH) as an independent disease entity.[5]

A French family, reported by Kaplan and associates,[6] and three American families studied by Egan and Klein,[7] appeared to have AEH. French, Danish[8] and American reports have referred variously to AEH as a "hyperkalemic variant" of familial periodic paralysis, familial periodic adynamia and hyperkalemic familial periodic paralysis.

It is the purpose of this communication to report studies of a patient with diabetes mellitus who is also thought to have AEH. Except for one individual in a family reported by Egan and Klein (but not available to them for study) we know of no other instance of these diseases co-existing in one individual.[9-11] The mother of the patient herein reported also has both diseases but was unavailable for study.

Presented at the Twentieth Annual Meeting of the American Diabetes Association in Miami Beach on June 12, 1960.

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CASE REPORT

M.G., aged thirty-three years, had had known diabetes mellitus for three years. She was referred to one of us because diabetes control had been difficult despite a carefully weighed diet. Insulin reactions were frequent, but diabetic coma had never occurred. She was at this time taking 35 units of NPH insulin, and her diet consisted of 240 gm. carbohydrate, 90 gm. of protein and 100 gm. of fat. Subcutaneous fat atrophy had developed at some undetermined time after initiation of insulin therapy. She had never experienced edema, induration or erythema of these areas on her thighs.

Her past history revealed diphtheria at the age of five, uncomplicated scarlet fever and bronchial asthma. She had developed keloids of moderate degree following appendectomy and smallpox vaccination. There had been no pregnancies, and no contraceptives were used during her six years of marriage. Menstrual history was normal and childlessness was unexplained despite repeated careful study of the patient and her husband. She denied a past history of discolored urine, renal calculi or abdominal pain associated with attacks of paralysis.

She first developed attacks of "stiffness" and muscle weakness prior to five years of age. In her youth they most often involved her legs and often caused truancy. After menarche they gradually became less frequent. Attacks of bronchial asthma occurred during her teens and frequently would be accompanied by mild to moderate muscle weakness. She had two moderately severe attacks prior to the diagnosis of diabetes mellitus and, subsequently, the episodes became more frequent and severe.

The mild attacks were described as a sensation of weakness and stiffness, primarily of leg muscles, and varied in duration from one hour to one week. Severe attacks started as a "heavy" feeling of the waist, back and eyes. Progression of attacks led to inability to walk, turn over in bed or lift her arms. These symptoms usually lasted one to three hours. Severe attacks would frequently, but not invariably, be followed by weakness lasting seven to ten days, noted particularly when attempting to climb stairs or lift heavy objects. Paralysis occurred most often in the fall and in damp weather, and seemed to develop in most instances while she was inactive after physical exertion. Emotional upsets apparently made the patient more prone to attacks. With adequate warning many episodes of paralysis were aborted by walking, and on several occasions the patient had walked continuously for two to three hours. The patient recalled a few instances when there was difficulty relaxing her

grip; however, there were no symptoms suggesting cold allergy or the "facial stiffness" of paramyotonia which develops subsequent to exposure to cold temperatures.

The family history revealed that her mother, sister and a paternal cousin had diabetes mellitus. Her mother also had bronchial asthma, episodic weakness and subcutaneous fat atrophy. There was no history of goiter or other endocrinopathy. No episodic paralysis or weakness was present on the paternal side of the family. The mother's family was found to contain sixteen members with episodic paralysis.

Physical examination revealed the weight to be 125 pounds, height 64 inches, temperature 98.6° F., pulse rate 80 and blood pressure 118 systolic and eighty-eight diastolic. She was well-developed and feminine and the skin was moderately dry. Small pearl-colored keloids were present in appendectomy and vaccination scars. The anterior thighs revealed typical subcutaneous fat atrophy. The remainder of the physical examination was not remarkable. A neurologic examination was not remarkable, and no myotonic reflexes were elicited at this time.*

On Feb. 22, 1958, a spontaneous attack developed which was described as moderately severe and the patient was reevaluated in a hospital. She was anxious and flushed, her blood pressure was 122 systolic and eighty diastolic, and pulse rate eighty-four. She was unable to stand or extend the extremities. All deep tendon reflexes were obtainable but hypoactive. Hoffman and Babinski reflexes were negative. The Chvostek sign was positive, the Trousseau negative. The grip was weak. Sensation was normal. Laboratory studies revealed a blood sugar of 383 mg. per 100 ml., calcium 5.2, potassium 7.8 and magnesium 2.0 mEq. per liter. The administration of calcium gluconate and magnesium sulfate had no apparent effect on her clinical state. Another spontaneous attack occurred on April 3, 1958, when insulin and breakfast had been withheld for an intravenous pyelogram. She developed moderate stiffness and "uneasiness." The potassium was 6.4 mEq. per liter. The fasting blood sugar done two and one-half hours previously was 368 mg. per 100 ml. Several hours later she denied any residual symptoms. Serum potassium determinations done the preceding and subsequent days were within normal limits.

When it was first determined that paralysis was associated with hyperkalemia, questionnaires were sent to members of the family. In all, sixteen members of this family are thought to have AEH. The inheritance apparently is autosomal dominant with complete penetrance with eleven females and five males affected as shown in figure 1. This family migrated to America from County Kerry, Ireland. Other reported national origins have included Sweden, France, Italy, Ireland and the Netherlands.

METHODS

Analyses for serum sodium, potassium, and calcium were performed by a Beckman D. U. spectrophotometer with flame attachment.[12] Erythrocyte potassium concentration was determined on lysed red cells following careful washing. Although no corrections were made for errors caused by trapped plasma in packed cells, these determinations are thought valid enough to show the trend or direction of potassium movement. Other laboratory tests utilized standard methods.

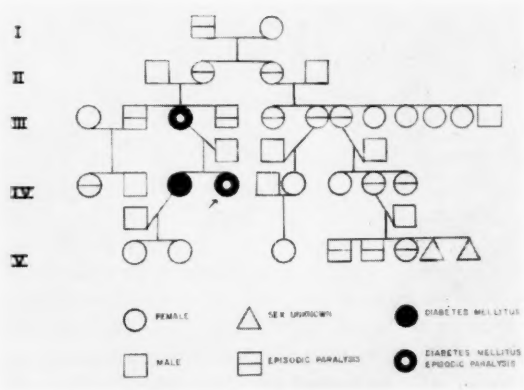

FIG. 1. Pedigree of family of M.G. showing the sixteen members (five males and eleven females) who are afflicted with episodic paralysis and weakness. The inheritance is autosomal dominant.

LABORATORY STUDIES

Laboratory investigation of M.G. during hospitalization (when she was symptom-free) disclosed normal findings for complete blood count, multiple urinalyses, sedimentation rate, Kline test for syphilis, protein-bound iodine, serum protein electrophoresis, serum sodium, potassium, chloride, calcium and carbon dioxide content. The phosphorus was 4.1 and cholesterol 286 mg. per 100 ml. The twenty-four-hour excretion of creatinine was 1.9 gm. and of creatine 38 mg. The urea clearance was 82 per cent of average normal clearance. The urinary excretion of pituitary gonadotropin, 17-ketosteroids (17-KS), 17-hydroxycorticoids (17-OH) and aldosterone was normal.* Corticotropin stimulation caused a normal increase in 17-KS and 17-OH. When dietary sodium had been maintained at 12.8 mEq. per day for ninety-six hours the sodium excretion decreased to 12 and the chloride to 16 mEq. per twenty-four hours. The potassium excretion was 46 mEq. per twenty-four hours. The addition of desoxycorticosterone (15 mg. per day for ninety-six hours) with continued salt restriction caused a reduced urinary volume and a slight reduction of serum potassium (4.0 mEq. per liter on two determinations); the urinary excretion of sodium was slightly increased, chlorides decreased and the potassium unchanged. Four days later with no salt restriction or medication the urinary volume nearly doubled, and the excretion of sodium

* Frank L. Engel, M.D., Durham, North Carolina, examined M.G. in November 1958, and elicited a "borderline" myotonic reflex in the thenar eminences.

* The aldosterone excretion was 10 μg. per day and the authors wish to thank Dr. Ralph E. Peterson, Bethesda, Maryland, for performing this test.

was 115, chloride 89 and potassium 34 mEq./L. An electrocardiogram under normal conditions revealed no significant abnormalities.

Chest and skull X rays revealed no abnormalities, and an intravenous pyelogram was normal. A spina bifida occulta was present (first sacral vertebra).

PRECIPITATION OF PARALYSIS

Gamstorp, et al. have stressed the diagnostic significance of precipitation of paralysis following potassium in AEH.[12] Accordingly, on April 26, 1958, the patient had her usual breakfast and insulin (32 units of Lente insulin). Four hours later 5.0 gm. of potassium chloride was ingested. Forty-five minutes thereafter a Chvostek reflex was observed which remained for 110 minutes. No changes were observed in pulse rate, blood pressure, cranial nerve function, sensation, or deep tendon reflexes, and the patient experienced no unusual symptoms. Frequent electrocardiograms and blood studies showed no significant abnormalities. One week later, breakfast and insulin were withheld, and at 9 a.m. the patient ingested 5.0 gm. of potassium chloride solution as before. Within fifteen minutes she experienced "heaviness" of the back and a "quivering" sensation of her eyes, and by 9:45 a.m. virtually complete flaccid paralysis. The blood pressure was 112 systolic and ninety-two diastolic. Speech was slurred, but extraocular movements were normal and there was no facial paralysis. The Chvostek sign was again present. No deep tendon reflexes were elicited, but superficial touch and vibratory senses were intact. The plantar response was normal.

Because of marked changes in the electrocardiogram (figure 2) 15 units of Regular Insulin was given intravenously at 10:20 a.m. At 10:25 a.m. her speech improved. By 10:33 a.m. she was able to flex her arms, rotate her extremities and move her toes. At 10:55 a.m. her speech seemed normal, arms could be elevated and knees could be raised two inches from the bed. At 11:06 a.m. the triceps reflex was 2+ bilaterally, the first deep-tendon reflex detected for a period of eighty-one minutes. In the next hour she became able to walk, albeit stiff-legged and with help. Correlated changes of serum and red cell potassium and electrocardiographic changes are shown in figure 3. The urinary excretion of sodium, potassium and chloride during this period was unchanged from the excretion during the preceding twenty-four hours.

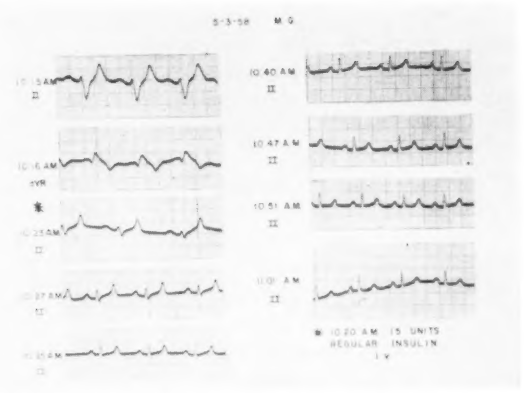

FIG. 2. Serial electrocardiogram during paralytic and recovery phase. Note rapid reversion to normal pattern after insulin administration.

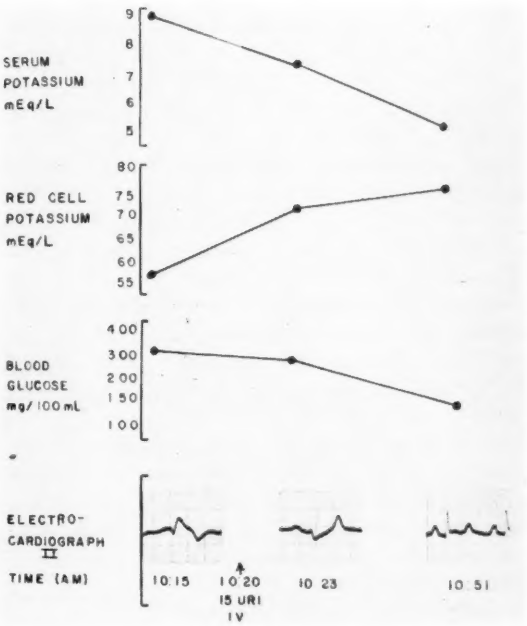

FIG. 3. Composite graph of serial changes of serum potassium, red cell potassium, and blood glucose concentrations correlated with electrocardiographic changes during paralysis and recovery. Note the apparent reciprocal changes between serum and red cell potassium values.

MISCELLANEOUS STUDIES

Egan and Klein[7] reported glucagon and epinephrine to be effective in lowering the elevated potassium values during induced attacks in nondiabetic individuals with AEH. Attacks of paralysis were prevented or of less severity and frequency when their patients with AEH were given dextro-amphetamine. In our patient, epinephrine was not evaluated, but Crystalline Insulin aborted virtually all spontaneous attacks of paralysis. Glucagon-free insulin, though deemed unsafe for the treatment of severe induced attacks, was made available

to the patient for use in treating spontaneous attacks, but data are incomplete at this time.*

Although not eliminating paralysis in our patient, the daily administration of dextro-amphetamine (15 mg., in long-acting form) has decreased the frequency and severity of attacks. It also has shortened recovery time and virtually eliminated lasting "hangover" symptoms of stiffness and weakness.

The details of this interesting disease are well reported in other studies and not pertinent to this report. The effect of anesthesia and unusual attacks occurring in older individuals of this family are to be reported elsewhere. Because every patient felt that anxiety increased the likelihood of attacks they had all been told that the illness was "functional" and due to nervous tension.

DISCUSSION

Two patients are reported with diabetes mellitus and associated episodic paralysis thought to represent AEH. Each, also, has subcutaneous fat atrophy. Few metabolic or endocrine diseases have been observed in reported families with AEH. One toxic goiter was reported by Gamstorp,[5] but thyroidectomy apparently had no effect on attacks of paralysis.

Our studies of one patient with diabetes mellitus and apparent AEH demonstrate normal renal, thyroid, pituitary and adrenal function. The increase in serum potassium during paralysis apparently is due to a shift of potassium to the extracellular space or a defect in the transfer of potassium to the intracellular space. That recovery from paralysis is associated with a shift of potassium to the intracellular space following administration of insulin is suggested by our data. No erythrocyte potassium determinations were accomplished during spontaneous attacks. In the reported instances of AEH potassium administration virtually always precipitated paralysis. When insulin and food were withheld potassium administration caused our patient to develop flaccid paralysis, areflexia, hyperkalemia and alarming electrocardiographic changes.

These data suggest that the usually benign nature of AEH may be altered when there is co-existent diabetes mellitus. However, paralysis was not precipitated by 5 gm. of KCl when the usual insulin and breakfast were given in the morning, whereas the same amount of KCl did precipitate paralysis when insulin and breakfast were withheld. Careful diabetes control had some effect in decreasing the frequency of paralytic attacks. By the administration of Crystalline Insulin the patient was able to abort virtually all episodes of paralysis. Symptoms of hypoglycemia never developed prior to the relief of the attacks. Glucagon-free Crystalline Insulin might be anticipated to be of less benefit than Crystalline Insulin in aborting paralytic episodes, since Elrick et al.[34] have reported that in diabetic patients a mixture of insulin and glucagon caused a greater glucose uptake by skeletal muscle than did insulin alone. In our patient the use of glucagon-free insulin is being evaluated for the treatment of spontaneous attacks of paralysis.

Dextro-amphetamine decreased the frequency of attacks and shortened the recovery period. Postparalytic symptoms of weakness and muscular stiffness were virtually eliminated. The mechanism of the prophylactic benefit of this drug is not understood, and too few patients have been so treated to warrant conclusions based entirely on subjective and historical data.

Recent evidence has suggested that paramyotonia congenita is a disease entity separate from myotonia congenita, and lability of serum and tissue potassium has been established.[35] However, attempts to precipitate paralysis by potassium administration have been unsuccessful.[9] Although similarities exist, available evidence does not warrant the conclusion that AEH and paramyotonia are the same disease.

CONCLUSIONS

Two patients are reported who have diabetes mellitus and hereditary episodic paralysis and hyperkalemia which is thought to represent adynamia episodica hereditaria. Studies of one of these individuals establishing normal renal, thyroid, adrenal and pituitary function are presented. The effects of administration of potassium, insulin, desoxycorticosterone and dextro-amphetamine are reported. The data suggest that the usual benign course of AEH may not obtain when there is associated diabetes mellitus, and that diagnostic attempts to precipitate paralysis by potassium administration should be performed with great care.

SUMMARIO IN INTERLINGUA

Episodic Paralyse Hyperkaliemic: Reporto de un Caso de Adynamia Episodic Hereditari Associate con Diabete Mellite

Es reportate duo patientes qui ha diabete mellite e paralyse episodic hereditari e hyperkaliemia interpretate como adynamia episodic hereditari. Es presentate studios in un de iste individuos establiente le presentia de un function normal de renes, thyroide, adrenales, e pituitario. Le effecto del administration de kalium, insulina, disoxycorticosterona, e dextro-amphetamina es reportate. Le datos suggere que le usualmente benigne curso de adynamia episodic hereditari es possibilemente non de regula in le presentia de un associate diabete mellite

* Glucagon-free insulin kindly furnished by W. R. Kirtley, M.D., Eli Lilly and Company, Indianapolis, Indiana.

de maniera que essayos diagnostic de precipitar paralyse per medio del administration de kalium debe esser interprendite con le plus grande circumspection.

ADDENDUM

Van Der Meulen, Gilbert, and Kane,[16] have reported a family who demonstrated contraction and percussion myotonia, and in whom the oral administration of moderate amounts of potassium precipitated flaccid muscular paralysis. No associated diabetes mellitus was reported. The quantity of potassium which was necessary to provoke paralysis was, in several instances, larger than recommended by Gamstorp[5] and reported by other authors. It seems likely that there exists a group of related hereditary syndromes of neuromuscular dysfunction associated with disturbances of potassium transfer.

ADDENDO

Van der Meulen, Gilbert, e Kane ha reportate le caso de un familia qui manifestava myotonia de contraction e percussion e in qui le administration oral de moderate quantitates de kalium precipitava un flaccide paralyse muscular. Nulle associate diabete mellite esseva reportate. Le quantitate de kalium necessari pro provocar le paralyse esseva in plure casos plus grande quo illo recommendate per Gamstorp e que illo reportate per altere autores. Il pare plausibile supponer que il existe un gruppo de relationate syndromes hereditari de dysfunction neuromuscular associate con disturbationes del transferimento de kalium.

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On Teaching Diabetes

Laboratory Work on Diabetes in the Basic Science Departments

In the endocrine subject committee at Western Reserve a number of interesting basic science laboratory experiments related to diabetes and to endocrinology were set up. These areas are particularly well suited to integrated presentation, since there is no clear separation between basic science and clinical science presentations. Although the basic scientist works primarily with animals while the clinical scientist works with man, both are concerned with the same general problems.

Now, in a subject such as anatomy a significant fraction of the material is best studied by direct laboratory observation. Thus, every medical student ought to study the component parts of the heart in the laboratory. By contrast, in subjects such as biochemistry or physiology, the basic information is acquired by the medical student through books and through lectures. The laboratory work in these subjects usually represents a sampling of typical experiments selected to demonstrate methodology and to provide a more critical basis for evaluating and interpreting information in the textbooks. Many different types of laboratory experiments will provide this experience, and therefore it is not essential that all medical students carry out the same laboratory experiment. This is particularly true in the field of endocrinology.

Let me illustrate some of the typical endocrine experiments that have been devised. These enable the medical student to observe directly and under controlled

(*Continued on page 469*)

Ultrastructural Changes in Islets of the Rat Produced by Tolbutamide

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St. Louis

The results of several different studies now indicate that the hypoglycemic effect of sulfonylurea compounds is apparently mediated by the release of increased amounts of insulin from the pancreas.[1-10] Yalow et al.[2] have demonstrated with the immunochemical assay for insulin that the administration of tolbutamide to normal human subjects produces an increase in the level of serum insulin. Pfeiffer et al.,[3] using the epididymal fat pad assay, have reported an increase of insulin-like activity of serum from normal patients and elderly diabetics and of serum from rats after treatment with sulfonylurea compounds. Morphological evidence of accelerated insulin release after sulfonylureas is seen in degranulation of pancreatic beta cells.[4-7] Intact beta cells are necessary for this response as shown by the absence of a hypoglycemic effect of sulfonylureas following total pancreatectomy,[8,9] in alloxan-diabetic animals[10] and in most juvenile diabetics.[3]

The mechanism by which sulfonylureas increase release of insulin from beta cells is unknown at the present time. The accumulation of information concerning their mechanism of action is of basic importance in the study of the etiology and pathogenesis of maturity-onset diabetes in man. In these patients, sulfonylureas apparently release sufficient quantities of insulin to maintain normoglycemia whereas the elevated blood sugar present in their diabetic state fails to stimulate the release of adequate amounts of insulin from their beta cells. Therefore, the objective of the present study was to utilize electron microscopy in determining the changes produced in the ultrastructure of beta cells of rats following treatment with tolbutamide. In order to obtain a closer correlation of the functional status of the beta cells with their morphologic appearance, the insulin-like activity (ILA) of the serum and the insulin content of the pancreases of the treated animals were measured.

Presented at the Twenty-first Annual Meeting of the American Diabetes Association in New York City on June 24, 1961.

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MATERIALS AND METHODS

Tolbutamide,* 150 mg./kg., was given either subcutaneously or in the femoral vein to 200-250 gm. male Wistar rats. A freshly prepared 10 per cent solution of tolbutamide in normal saline was used for all injections. Thirty animals received a single intravenous injection of tolbutamide and were killed in groups of four or five at intervals of 10, 30, 60, 120, and 180 minutes. Another fifty animals each received either one or two subcutaneous injections of tolbutamide; the second injection was given eight hours after the first. The animals were then killed in groups of four or five at intervals of 1, 3, 4, 8, 9, 12, 24, 32, and 48 hours after the first injection. Fifteen animals served as controls.

All animals were anesthetized with nembutal, and blood was removed from either the portal vein or the inferior vena cava. Specimens of blood were kept at room temperature for two to three hours, then centrifuged and the serum was removed. Serum sugars were determined by the ferric-ferrocyanide method[11] using an auto-analyzer. The epididymal fat pad technic as described by Renold et al.[12,13] was used to determine the insulin-like activity (ILA) of the serum which was diluted 1:4 or 1:6 prior to assay.

Portions of the pancreas from two or three animals of each group were removed rapidly and prepared for light and electron microscopy. The tissue for electron microscopy was fixed in 1 per cent osmic acid dichromate solution buffered to *p*H 7.6,[14] dehydrated through a series of graded alcohols to absolute ethanol and embedded in methacrylate. Thin sections of the pancreas were stained with lead acetate[15] prior to examination with RCA electron microscopes models 3C and 3E. Electron

* Tolbutamide (Orinase) was a generous gift of Dr. Macleod of The Upjohn Company.

micrographs were taken at initial magnifications of 1,500 to 11,000 X and then enlarged photographically as desired. The pancreatic tissue for light microscopy was fixed in Zenker-formol and embedded in paraffin. Sections were cut at 5 μ and stained with aldehyde fuchsin[18] and hematoxylin and eosin.

The entire pancreas of three or four rats of each group was removed for insulin extraction. The pancreases were carefully dissected from the duodenum, stomach, and surrounding structures in order to remove all the pancreatic tissue possible. They were then weighed, frozen in liquid nitrogen and stored at —20° C. Each pancreas was later thawed in 10 ml. of acid alcohol,* cut into small pieces, extracted for a period of two hours at room temperature, filtered through gauze and then was re-extracted with the same volume of acid alcohol for a second period of two hours. The volume of the combined acid alcohol extracts was measured and stored at —20° C. until they were assayed for insulin. The insulin content was determined with the epididymal fat pad technic by adding aliquots (7.57 μl) of the extracts to each assay bottle. The total insulin content of the pancreas was calculated and expressed as units of insulin per 100 gm. of body weight.

RESULTS

The ultrastructure of the normal islet cells of the rat has been described previously.[17-21] The cytoplasm of the normal beta cell contains an abundance of lamellar ergastoplasm as well as particulate ribonucleoprotein granules, mitochondria, and scattered collections of smooth membranous sacs of the Golgi complex (figures 1 and 2). Beta granules are numerous and appear as moderately dense, round structures enclosed in smooth-walled membranous sacs with a variable space separating them. With higher powers of magnification, the internal structure of some of the beta granules has a vesicular or porous appearance (figure 3), whereas others are homogeneous. Occasional cytoplasmic processes (microvilli) project from the surface of beta cells into pericapillary and intercellular spaces.

By light microscopy slight degranulation of beta cells and margination of the remaining granules were evident thirty to sixty minutes after a single intravenous injection of tolbutamide. The earliest change observed in electron micrographs of beta cells was an apparent margination of granules with their surrounding sacs, to the surface of the cell (figure 4). This change was

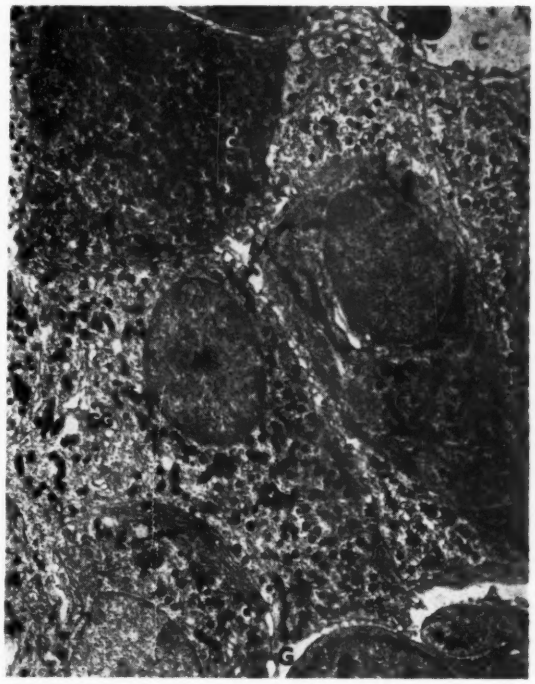

FIG. 1. Normal beta cells in an islet of Langerhans of the rat. Many beta granules (G), lamellar ergastoplasm (E), Golgi vesicles (GG), and mitochondria (M) are present in the cytoplasm. Nucleus (N), capillary lumen (C). X 5,000.

evident ten minutes after a single intravenous injection of tolbutamide. As the beta granule with its enveloping sac approached the plasma membrane the latter two structures apparently fused (figures 4, 5, 6) and then burst, releasing the granule into the extracellular space. The released granule apparently undergoes rapid dissolution in the extracellular fluid since we did not observe either intact granules or portions of them outside the beta cells. Figure 7 illustrates the outline of a space previously occupied by a beta granule just after it has been released from the cell. The basement membrane is slightly bulged over this space and forms its outer limit. The remnant of the membranous sac encasing the granule has apparently become continuous with the plasma membrane of the cell. This process of ejecting beta granules into the extracellular space has been termed emiocytosis.[22]

The margination of beta granules along plasma membranes was followed by the appearance of increased numbers of microvilli projecting into the intercellular and pericapillary spaces (figure 8). The formation of these microvilli appeared to be related to the release

* 95 per cent ethyl alcohol, 790 ml.; conc. HCL, 15 ml.; H_2O, 195 ml.

FIG. 2. Microvilli (V), beta granules (G), lamellar ergastoplasm (E), particulate ribonucleoprotein granules (R), Golgi vesicles (GG), and mitochondria (M) are demonstrated at higher magnification in portions of two normal beta cells. Capillary lumen (C), pericapillary space (PS). X 32,000.

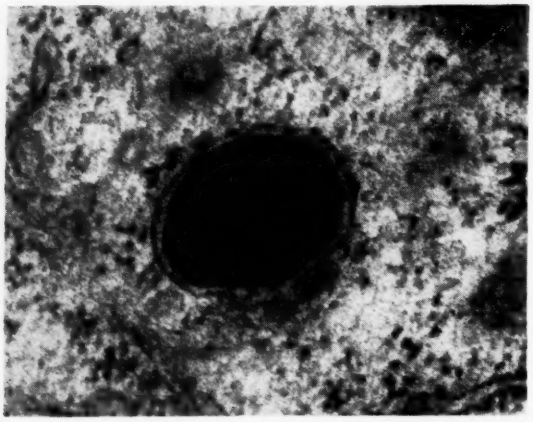

FIG. 3. The porous or vesicular appearance of a beta granule is shown at high magnification. X 100,000.

of beta granules. The cytoplasm on either side of the space previously occupied by the granule remained in the form of short processes or microvilli (figures 6 and 7). The continued release of beta granules at this same focus and adjacent to these processes would therefore result in the formation of even longer microvilli.

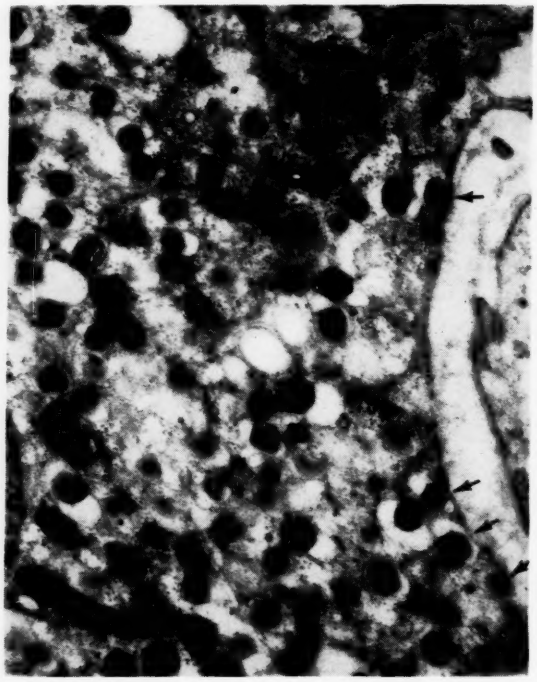

FIG. 4. Margination of beta granules to the plasma membrane (↑) is shown in an animal nine hours after the first of two subcutaneous injections of tolbutamide given eight hours apart. Some of the membranous sacs surrounding the granules have fused with the plasma membrane. X 28,000.

Slight to moderate degranulation of beta cells was evident with both light and electron microscopy at one and four hours and was more prominent at eight hours after a single subcutaneous injection of tolbutamide. Degranulation of beta cells was further increased by the second injection of tolbutamide and was most advanced at twenty-four hours (figures 9, 10, and 11). At thirty-two hours, granulation was normal in some animals and slightly decreased in others and at forty-eight hours it was essentially normal in all the pancreases examined.

With electron microscopy, a distinct change was observed in the ergastoplasm of beta cells following the second injection of tolbutamide. In the normal beta cell the ergastoplasm is composed of closely applied lamellae with attached ribonucleoprotein granules on their outer surfaces (figure 2). In some of the partially or completely degranulated cells observed at twelve and

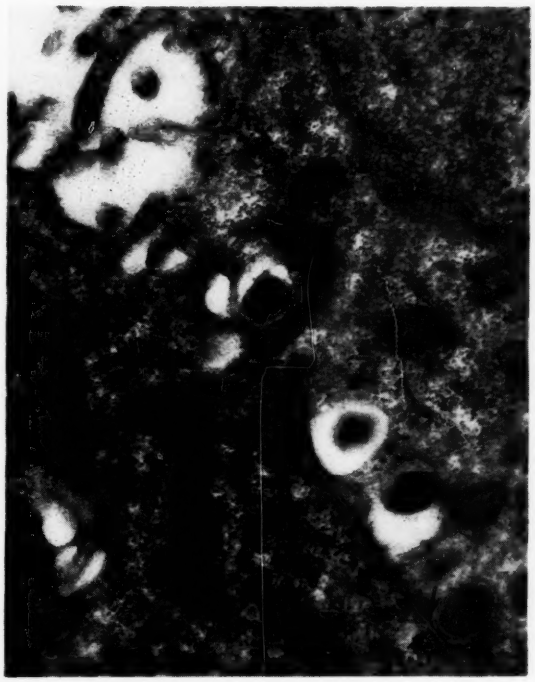

FIG. 5. Margination of beta granules (↑) in an animal twelve hours after the first of two subcutaneous injections given eight hours apart. The membranous sac of the granule in the right lower corner is separated from the plasma membrane (P) by a distinct space. Sacs of the granules above it are partially fused with the plasma membrane. X 36,000.

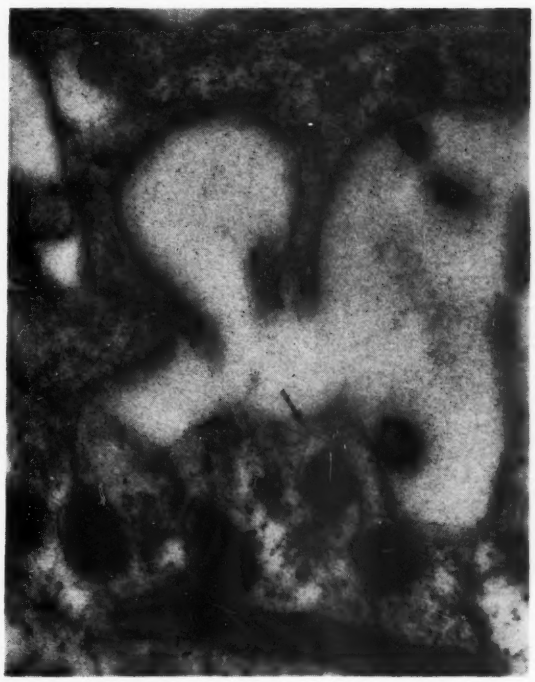

FIG. 6. Fusion of the membranous sac of a granule with the plasma membrane is shown at the arrow (↑). The cytoplasm to the right of the granule has the general appearance and configuration of a microvillus. X 55,000.

twenty-four hours the ergastoplasm appeared vesicular instead of lamellar (figures 12 and 13). The outer surfaces of these vesicles had attached ribonucleoprotein granules and the walls of some had an irregular contour, whereas others appeared circular. The lumen of these vesicles contained a pale amorphous material (figure 13). In some, distinct beta granules were observed with ribonucleoprotein granules still evident on their outer surfaces (figure 14). At thirty-two hours, the cytoplasm of the beta cells was filled with distinct beta granules which were enclosed in smooth membranous sacs and the ergastoplasm had a lamellar appearance similar to that of the normal beta cell. These findings suggest that the pale grey amorphous material observed in the ergastoplasmic vesicles represents an early stage in the formation of beta granules, and that it subsequently coalesces into the dense homogeneous structure of the mature granule. The ribonucleoprotein granules present on the outer surfaces of these vesicles are apparently lost as the granule matures thus leaving it encased in a smooth membranous sac.

The serum sugar levels of portal vein blood decreased rapidly to 30 per cent of the control value one hour after a single intravenous injection of tolbutamide and subsequently returned to 70 per cent of the control values at three hours (figure 15). The serum ILA was double the base line values at ten minutes and then fell rapidly to below normal at thirty and sixty minutes. The statistical probability of the differences between the baseline and thirty-minute means and the mean at ten minutes being due to chance is less than 1 per cent. The values were slightly above control levels at two and three hours after the injection.

After a single subcutaneous injection of tolbutamide, both the peripheral and portal serum sugar levels decreased to approximately 50 per cent of the control values at one hour, remained at about the same level at two hours and then returned to normal three and four hours after the injection (figure 16). A similar depression, although of greater magnitude (30 per cent of control levels), occurred one hour after the second injection of tolbutamide. A gradual increase in serum ILA of peripheral venous blood occurred during a four-hour period after the first injection and a similar

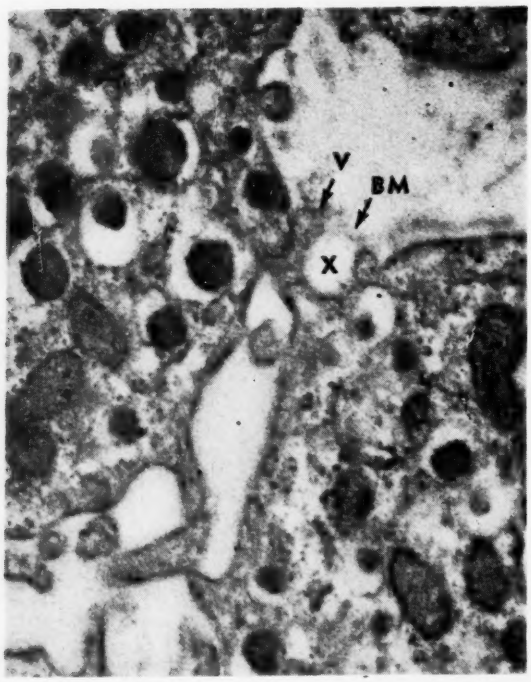

FIG. 7. (Left) The space previously occupied by a granule contains an X. Basement membrane (BM) overlying the opening of the sac is slightly bulged. The cytoplasm to the left of the space previously occupied by the granule remains in the form of a microvillus (V). This animal was killed fifteen minutes after receiving an intravenous injection of tolbutamide. X 35,000.

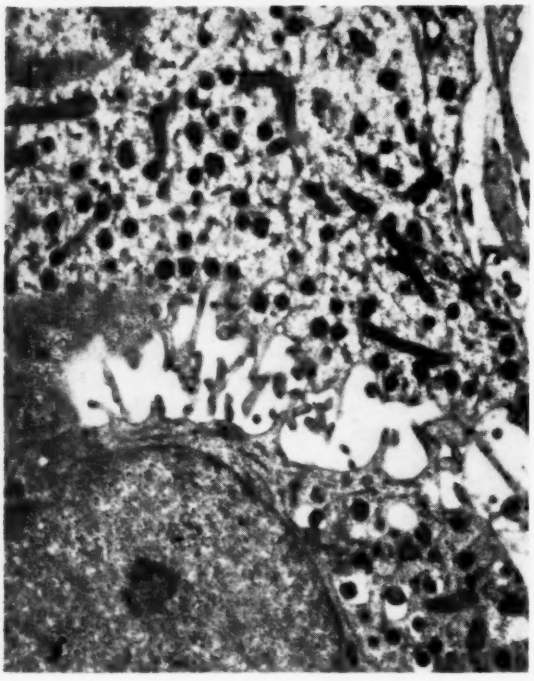

FIG. 8. (Left) Numerous microvilli project into the intercellular space between two beta cells from an animal nine hours after the first of two subcutaneous injections given eight hours apart. X 12,000.

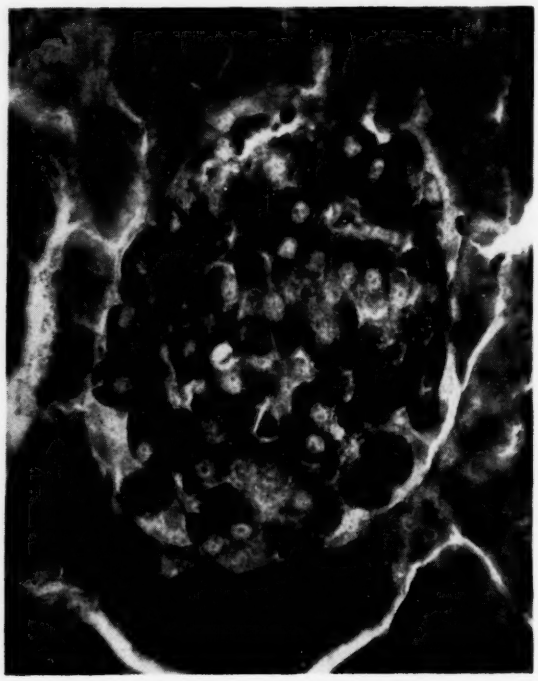

FIG. 9. (Above) Islet of Langerhans from a normal rat. Aldehyde fuchsin. X 690.

increase occurred during the four-hour interval following the second injection of tolbutamide. The only detectable increase in ILA of portal serum was four hours after the first subcutaneous injection of tolbutamide.

The insulin content of the pancreases obtained at different intervals after subcutaneous injections of tolbutamide are shown in table 1. At eight hours, the extractable insulin from the pancreas was only 50 per cent of the control values. The amount of insulin lost from the pancreas during this interval was approximately 0.5 unit. The lowest amount of extractable insulin from the pancreas was found at sixteen hours after the second injection. Subsequently the insulin content returned toward the normal control levels.

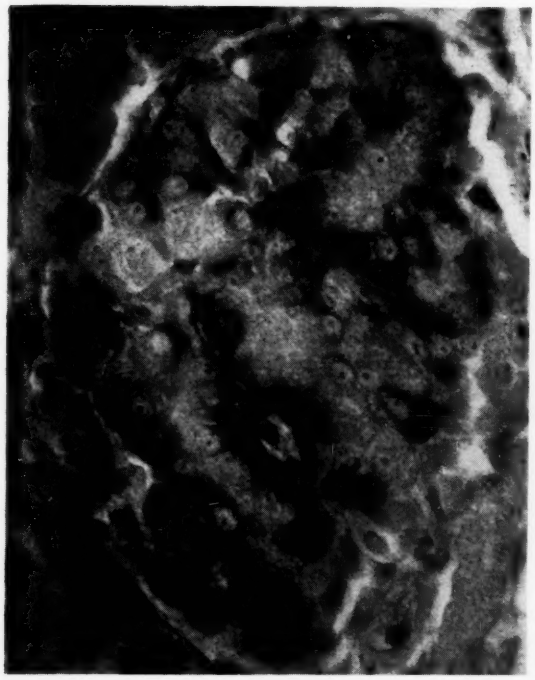

FIG. 10. (Left) Degranulation of beta cells with margination of remaining granules in an islet of Langerhans twelve hours after the first of two subcutaneous injections of tolbutamide given eight hours apart. Aldehyde fuchsin. X 690.

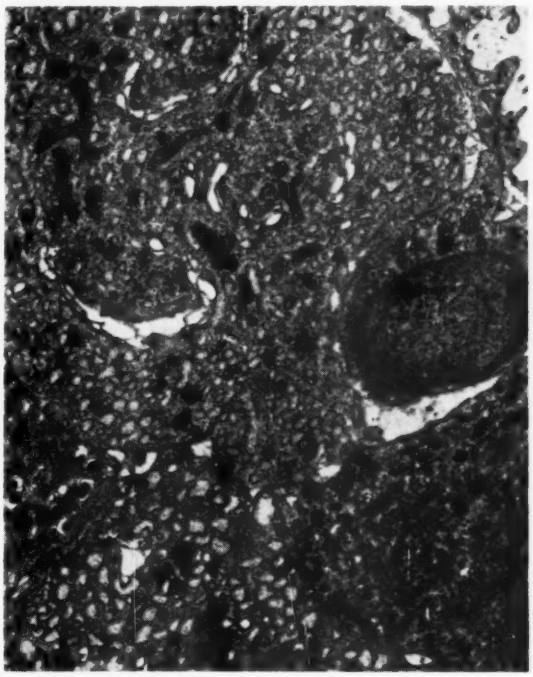

FIG. 12. (Above) Dilatation and vesiculation of ergastoplasm of degranulated beta cells twelve hours after the first of two subcutaneous injections of tolbutamide given eight hours apart. X 14,000.

DISCUSSION

The ultrastructural changes observed in beta cells following the administration of tolbutamide consist of (1) degranulation, (2) vesiculation of ergastoplasm, and (3) regranulation. Beta granules are released from the cells by a process of ejection or emiocytosis. The beta granule with its surrounding membranous sac appears to migrate to the surface of the cell. The sac then fuses with the plasma membrane and ruptures, releasing the granule into the extracellular fluid where it rapidly undergoes dissolution. A similar mechanism for the release of secretory granules has been observed in cells of the anterior pituitary by Farquhar.[23] The re-

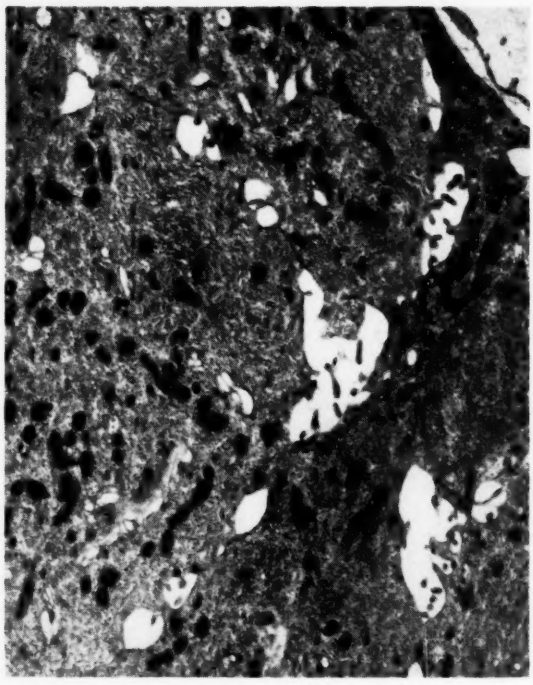

FIG. 11. (Left) Marked degranulation of beta cells and increased prominence of intercellular spaces and microvilli twenty-four hours after the first of two subcutaneous injections of tolbutamide given eight hours apart. X 9,000.

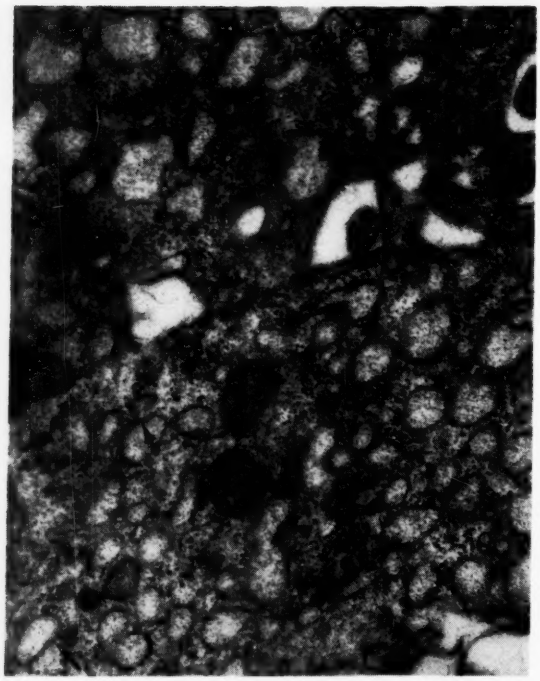

FIG. 13. A higher magnification of figure 12 to demonstrate the pale amorphous material in ergastoplasmic vesicles and ribonucleoprotein granules attached to their outer surfaces (↑). X 34,000.

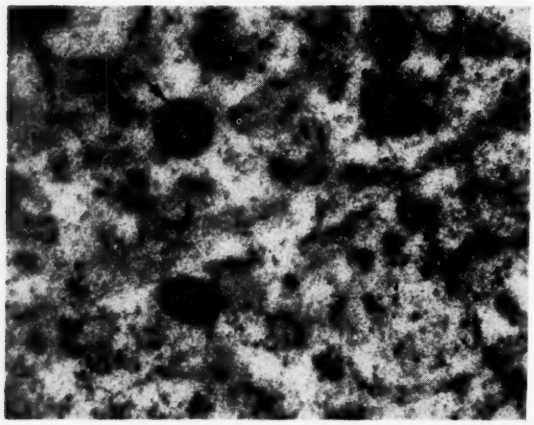

FIG. 14. A beta granule (↑) with ribonucleoprotein granules attached to the outer surface of its sac twenty-four hours after the first of two subcutaneous injections of tolbutamide. X 45,000.

lease of beta granules by this method also results in the production of microvilli which extended into the intercellular and pericapillary spaces. This same mechanism of secretion has been observed following the induction

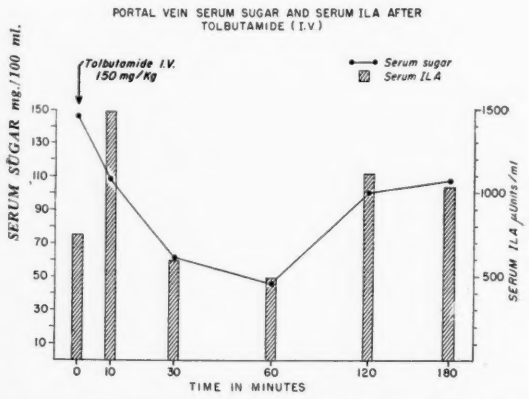

FIG. 15. Portal vein serum sugars and insulin-like activity after injection of tolbutamide, 150 mg./kg., into the femoral vein.

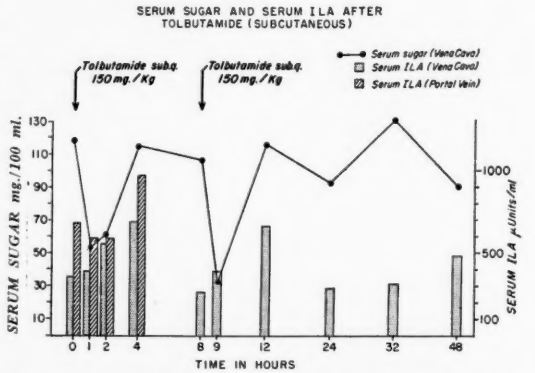

FIG. 16. Portal vein and vena caval serum sugars and insulin-like activity after a single subcutaneous injection of tolbutamide, 150 mg./kg., and after two subcutaneous injections given eight hours apart.

of a marked hyperglycemia in rats.[24] In addition, a similar mode of secretion apparently occurs in the normal beta cell of the rat since some microvilli are observed projecting from beta cell surfaces and occasionally a beta granule with its surrounding membranous sac can be observed immediately adjacent to the plasma membrane. These findings indicate that in the rat the same morphologic mechanism of beta cell secretion occurs following stimulation with tolbutamide as is found in normal beta cells and in beta cells stimulated by hyperglycemia.

The following observations in the present study support the findings of others[2-7] that tolbutamide accelerates the release of insulin:

(1) Degranulation of beta cells was evident by both light and electron microscopy.

TABLE 1

Insulin content of the pancreas after subcutaneous injection of tolbutamide (150 mg./kg.)

Time after first injection	Number of injections	Number of animals	Insulin content of pancreas (units/100 gm. body wt.)
Control	0	3	.440 ± .16*
8 hours	1	3	.258 ± .01
9 hours	2†	3	.200 ± .02
12 hours	2	4	.272 ± .18
24 hours	2	3	.167 ± .02
32 hours	2	4	.294 ± .22
48 hours	2	3	.281 ± .02

* Standard error of the mean.
† Second injection given eight hours after the first.

(2) The extractable insulin content of the pancreas was markedly reduced.

(3) An increase in serum insulin-like activity could be demonstrated following the administration of tolbutamide.

The site of action of tolbutamide may be on the beta granules or the cell surface, or it may affect the normal regulatory mechanisms which control insulin release. The lack of any alteration in the ultrastructure of the beta granules suggests that tolbutamide has not produced any marked change in the structure of the granules; however, this does not eliminate the possibility that it may in some way alter the physical state of insulin within the secretory granules. The effect of tolbutamide on insulin synthesis has not been determined. The degranulation of beta cells and the depletion of pancreatic stores of insulin for as long as sixteen hours after two subcutaneous injections indicate at least that insulin release is increased proportionally more than insulin synthesis in the rat.

The site of formation of beta granules is apparently in the ergastoplasm of the cells. The morphologic evidence for this is manifested by the transformation of a lamellar type of ergastoplasm to a vesicular form and the presence of either amorphous material or distinct beta granules within the lumen of these vesicles or sacs. Recent morphologic and biochemical studies of Siekovitz and Palade[25,26] and the earlier electron microscopic studies of Weiss[27] indicate that the synthesis of zymogen granules of the pancreas occurs in the ergastoplasm. The amorphous material observed in the vesicular ergastoplasm of the beta cells probably represents a precursor of a biologically inactive form of insulin since the extractable insulin content of the pancreas was lowest when these vesicles appeared most prominent (sixteen hours after the second injection of tolbutamide). In addition, this material apparently did not stain with aldehyde fuchsin since the most marked degranulation of beta cells was observed at this interval.

The serum ILA of samples from the inferior vena cava was approximately double the control value four hours after the first and second subcutaneous injections of tolbutamide. The hypoglycemic response to tolbutamide was maximal at one hour and had returned to normal levels at four hours. The absence of hypoglycemia in the presence of an elevated serum ILA at four hours is probably due to compensatory hyperglycemia. That such compensatory mechanisms undoubtedly are important in returning the blood sugar to normal has been demonstrated by Houssay and Penhos[28] who have shown that the hypoglycemic response of adrenalectomized rats to tolbutamide is much more marked and of longer duration than that of the normal animal.

At eight and sixteen hours after the first and second injections respectively both the serum sugars and the serum ILA had returned to control levels. The insulin content of the pancreas on the other hand was reduced by almost 50 per cent at the first interval and by 64 per cent at the second. This decrease in extractable insulin from the pancreas, and the elevation of serum insulin-like activity after intravenous tolbutamide indicate that the primary effect of tolbutamide in the rat is to accelerate the rate of release of insulin rather than to inhibit processes involved in insulin degradation and that its effect on insulin release is proportionally greater than a possible effect, either primary or secondary, on insulin synthesis.

Further studies are needed in order to interpret the precise interrelationships of the tolbutamide induced hypoglycemia, serum ILA and the insulin content of the pancreas during the first few hours after treatment. The present findings on the serum ILA of the portal vein suggest that tolbutamide administered intravenously may induce an immediate release of insulin followed by a second increased loss of insulin from the pancreas three to four hours later. Additional observations are needed on the ILA of peripheral venous serum and the insulin content of the pancreas following an intravenous injection of tolbutamide as well as determinations on peripheral and portal serum ILA during the first hour after a subcutaneous injection before this biphasic response can be established.

SUMMARY

The effects of tolbutamide on the ultrastructure of pancreatic beta cells were studied in the rat. Degranulation and margination of remaining granules were

evident by both light and electron microscopy after a single intravenous injection of tolbutamide and were marked twenty-four hours after the first of two subcutaneous injections given eight hours apart. The granules were released by a process of emiocytosis in which the membranous sac enclosing the granule fused with the plasma membrane and then burst, releasing the granule into the extracellular space where it rapidly dissolved. Increased numbers of microvilli projecting from the surface of beta cells appeared to be formed from the cytoplasm which remained on either side of the space previously occupied by a beta granule.

Marked vesiculation of ergastoplasm occurred in many degranulated beta cells at twelve and twenty-four hours. Pale amorphous material in these vesicles, and beta granules enclosed in sacs with ribonucleoprotein granules on their outer surface suggested that the site of synthesis of beta granules is in the ergastoplasm.

Morphologic evidence of increased insulin release by tolbutamide was supported by increased levels of serum insulin-like activity, a decrease in extracted insulin from the pancreas, and hypoglycemia. These observations indicate that in the rat tolbutamide accelerates the rate of release of insulin from beta cells, and that its effect on insulin release is proportionally greater than a possible effect, either primary or secondary, on insulin synthesis.

SUMMARIO IN INTERLINGUA

Alterationes Ultrastructural, Producite per Tolbutamida in le Insulas de Langerhans del Ratto

Le effecto de tolbutamida in le ultrastructura de pancreatic cellulas beta esseva studiate in le ratto. Disgranulation e margination del remanente granulos esseva evidente in microscopia a lumine e electronic post un sol injection intravenose de tolbutamida. Illos esseva marcate vinti-quatro horas post le prime de duo subcutanee injectiones effectuate con un intervallo de octo horas. Le liberation del granulos progredeva per plure phases. Primo le sacco que includeva le granulo individual se fusionava con le membrana plasmatic e alora rumpeva, emittente le granulo ad in le spatio extracellular ubi illo se dissolveva rapidemente. Augmentate numeros de microvillos, projicite ab le superficie del cellulas beta, pareva formar se ab le cytoplasma que permaneva al lateres del spatio previemente occupate per le granulo beta.

Marcate grados de vesiculation de ergastoplasma occurreva in numerose disgranulate cellulas beta post intervallos de 12 e 24 horas. Pallide amorphe material in iste vesiculas e granulos beta includite in saccos con granulos de ribonucleoproteina al superficie exterior suggereva que le sito de synthese del granulos beta es in le ergastoplasma.

Indicationes morphologic de un augmento del liberation de insulina como effecto del tolbutamida esseva corroborate per augmentate nivellos seral de activitate insulinoide, un reduction del insulina extrahite ex le pancreas, e le presentia de hypoglycemia. Iste observationes demonstra que in le ratto tolbutamida accelera le liberation de insulina ab le cellulas beta e que su effecto in le liberation de insulina es proportionalmente plus grande que un effecto possibile—primari o secundari—in le synthese de insulina.

ACKNOWLEDGMENT

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Laboratory Work on Diabetes in the Basic Science Departments

(Continued from page 459)

conditions some of the phenomena which he usually only reads about in the textbooks or hears about at second hand from the patient:

In one instance a group of medical students is given a number of rats to observe for food intake, weight gain, water intake, and twenty-four-hour urine output. After the blood sugar level and urinary glucose output are studied for an appropriate period, some of the rats are injected with alloxan, which selectively kills the pancreatic beta cells. It is exciting for the student to follow the blood sugar changes in these animals and to discover that suddenly the rat begins to lose weight rapidly, even though his food intake has doubled or tripled. The student observes that the rat begins to drink enormous quantities of water—up to 160 cc. per day, and that a rat weighing only 150 gm. may put out 150 cc. of urine, containing 8 gm. of glucose. Thus the student can get a more dynamic and graphic understanding of diabetes early in his medical career, an understanding that will be useful to him in his clinical work.

Then, by injecting insulin into these diabetic rats, the students can observe the effects of insulin therapy on the blood sugar, urine glucose, weight, and water intake. By injecting an overdose of insulin, they can cause the blood sugar level to drop to hypoglycemic levels, and thus they can witness a hypoglycemic convulsion—something the medical student rarely observes in man. The student can inject various types of insulin —such as Regular, or Protamine Insulin—into the diabetic rat and then compare their respective time courses in lowering blood sugar levels. Or he may inject large doses of cortisone into normal animals, and having thus produced steroid diabetes, he can show that these animals have become strongly resistant to insulin therapy.

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Postnatal Growth of the Endocrine and Exocrine Parts of the Rat Pancreas

Its Relationship to the Metabolism of DNA

Bo Hellman, M.D., Claes Hellerström, M.L., and Birger Petersson, M.K., Upsala, Sweden

Characteristic differences have been demonstrated between the postnatal growth of the pancreatic islet organ and the pancreas as a whole.[1-4] While there was a linear relationship between the logarithms of the pancreatic and body weights, the corresponding regression between the logarithms of the islet volume or the number of islets and the body weight was curvilinear, so that the initial increase was relatively small, but in the higher age groups reached or exceeded that of the pancreatic weight. Since the initial rate of increase in the number and total volume of the islets was slow but later accelerated with age, the ratio between either of these two quantities and the pancreatic weight was high at birth, later becoming reduced to a minimum value, and subsequently rising again significantly in the older animals.

The data obtained on the net growth of the islet tissue and the exocrine parenchyma do not give direct information about the cell turnover rate at different ages in the rat. Our aim is therefore to complement the investigations mentioned above by recording autoradiographically the cell proliferation within the pancreas, as manifested by the DNA metabolism of the cells. The tritiated form of the specific DNA precursor thymidine, with its low energy β emission, is well suited for this purpose.

MATERIALS AND METHODS

Fourteen rats of the Wistar strain were injected intraperitoneally on the first and third days of life with ^{3}H-thymidine (specific activity 2.7 C/mM) in a dose corresponding to 1 μC/gm. body weight. After weaning, the animals were given free access to water and food (caloric composition: carbohydrate 38 per cent, fat 24.5 per cent and protein 37.5 per cent). They were killed by decapitation at different times between the fourth and 154th days of life (see table 1). The pancreatic glands were immediately dissected and fixed in 10 per cent neutral formalin. After dehydration and clearing, the organs were embedded in paraffin and cut into 5 μ thick sections. These were covered with Kodak AR 10 stripping film and exposed for seventy-five days at 4° C. The films were developed for five minutes in D19B solution and then stained with hematoxylin.

Those cells were denoted as labeled, where the number of grains over the nucleus was at least three more than the background value. For the majority of the animals the information regarding the percentage figure of labeled cells in the islet tissue was based on microscopic examination of twenty-five randomly selected islets (objective 100 ×, N.A. 1.32). In the two youngest age groups, however, an average of fifteen islets, corresponding to about 1,000 cells, were studied. Moreover, in each of the seven oldest animals the frequency of labeled cells was also estimated both centrally and peripherally in four large islets (diameter > 100 μ). For this purpose the central zone was defined as lying within a distance of half the islet radius from the center, the peripheral zone being the remainder of the islet.

The frequency of labeling in the exocrine parenchyma was studied with regard to whether the cells lay (a) close to the islets and (b) far from the islets, the arithmetic mean of the percentage numbers in the two regions being considered as representative of the exocrine tissue in its entirety. In the region near to the islets, all cells were included in those acini, which were in contact with at least ten randomly selected medium-size and large islet section surfaces. To obtain the percentage frequency of labeled cells far from the islets, regions were chosen at such a low magnification that it was possible to identify the islets but not the grains in the emulsion. Subsequently, sufficient visual fields were analyzed at a high magnification, so that the number of exocrine cells counted was at least 500.

The random error in the determinations of the frequency of labeled cells was assessed by comparing in each animal those values obtained when the islets had been grouped as pairs. Such an analysis showed that it

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was insignificant throughout except for the separate analysis of the central and peripheral regions within the large islets.

RESULTS

Cells with radioactive nuclei were found in both the exocrine and endocrine parenchyma in all age groups (see figures 1 and 2). The percentage number of labeled cells at different times after the administration of ^{3}H-thymidine is given in table 1 and figure 3. Simultaneously with a decrease in the average number of grains over the nuclei the initially high frequency of labeled cells in both the islets and the acinar tissue also diminished. The latter decrease was greater in the exocrine parenchyma. While the initial value for the exocrine regions was about 55 per cent, on the 154th day it was less than 7 per cent. The corresponding figures for the islets were about 45 per cent and 10 per cent respectively.

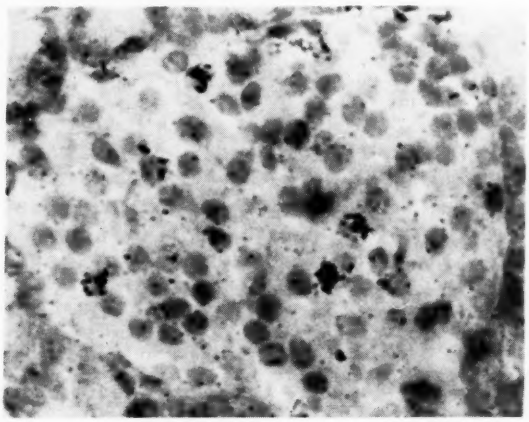

FIG. 1. Autoradiogram, showing an islet with labeled cells.

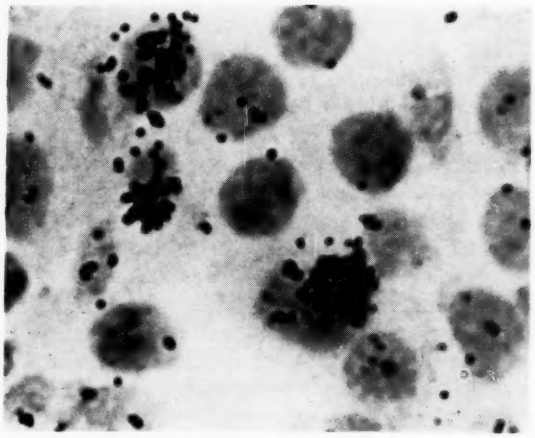

FIG. 2. High power view of an autoradiogram, showing three strongly labeled islet cells.

TABLE 1

The percentage number of labeled cells within the islets and the exocrine parenchyma in rats at different ages, which were injected on the first and third days of life with tritiated thymidine. The frequency in the exocrine parenchyma has been given with regard to whether the cells lie (1) close to the islets or (2) far from the islets. The difference between the percentage figures for the two regions in the animals concerned, is given in the right-hand column.

Animal	Age in days	Islets	I. Close to the islets	II. Far from the islets	Difference I-II
1	4	47.0	66.3	50.7	+5.6
2	4	44.0	54.9	47.4	+7.5
3	11	53.1	59.4	44.2	+15.2
4	11	50.0	55.7	44.9	+10.8
5	22	27.4	17.7	25.7	—8.0
6	22	22.8	23.7	24.7	—1.0
7	42	17.8	7.0	11.2	—4.2
8	42	21.7	6.7	11.7	—5.0
9	63	18.3	6.0	13.0	—7.0
10	63	15.8	6.9	11.1	—4.2
11	95	18.4	8.9	11.7	—2.8
12	95	8.7	4.6	6.3	—1.7
13	154	6.6	3.0	6.1	—3.1
14	154	14.4	6.7	10.8	—4.1

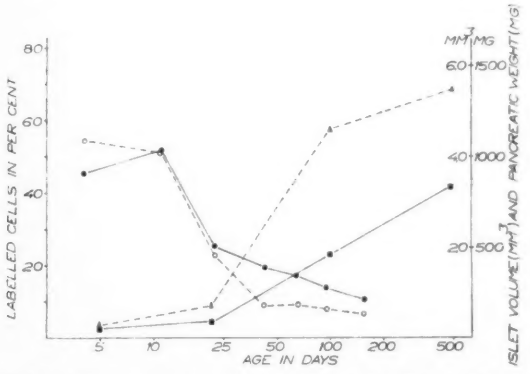

FIG. 3. The percentage number of labeled cells at different ages in the islet tissue (—●—●—) and the exocrine parenchyma (—○—○—). The arithmetic mean for the percentage number of cells lying close to and far from the islets respectively, has been regarded as representative of the exocrine tissue in its entirety. Every point represents the average value for two animals. Previously obtained values for the total islet volume in mm³ (—■—■—) and the pancreatic weight in mg. (—△—△—) for Wistar rats of different ages, have also been included as a comparison. Every point here represents a mean value of fifteen animals. The age of the rats in days is on a logarithmic scale.

Since the percentage number of labeled cells in the exocrine tissue is initially higher, but later becomes lower than the corresponding value for the islets, the curves in figure 3 intersect. In both tissues, the greatest decrease was obtained between the eleventh and twenty-second days of life. After the twenty-second

day, the percentage number of labeled cells in the islets decreases linearly (semilogarithmic scale) while that of the exocrine parenchyma decreases rapidly at first, but after the forty-second day of life flattens out. This tends to reduce the difference between the two curves.

Differences in the labeling frequency were noted between the exocrine cells localized near to the islets, and those which were found far from them (see table 1). The frequency of labeled cells near to the islets was initially 60 per cent, but had decreased to as little as 5 per cent by the 154th day of life. The corresponding values for the exocrine cells found far from the islets were 49 per cent and 8 to 9 per cent. Thus the curves expressing these values as a function of time (logarithmic) intersect, the curve for the cells found far from the islets assuming a relatively more horizontal course (see figure 4).

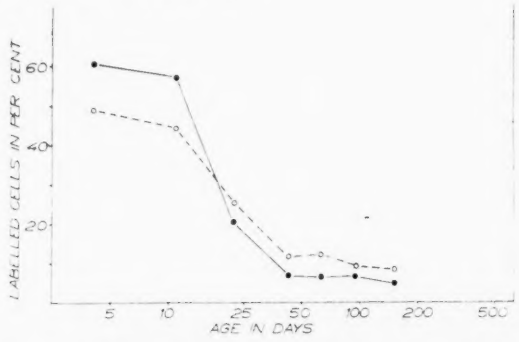

FIG. 4. The percentage number of labeled cells at different ages in the exocrine parenchyma near the islets (—●—●—) and far from the islets (—○—○—). Every point corresponds to two animals. The age of the rat in days is on a logarithmic scale.

No systematic differences in the labeling frequency between the central and peripheral parts of the large islets could be demonstrated (see table 2). It was difficult to assess, in the absence of selective differential staining of the autoradiograms, the distribution of labeled nuclei among the α and β cells. On the basis of the characteristic nucleo-cytoplasmic volume ratio of the α cells,[4] however, groups of indisputable α cells could in some cases be identified in the periphery of the islets, and it was possible to establish that a number of these cells had been labeled. Within the older age groups both the frequency of labeled α cells and their average degree of blackening seemed to be higher than for the β cells situated in the same islets.

TABLE 2

A comparison between the percentage frequency of labeled cells (1) peripherally and (2) centrally in the large islets in the seven oldest animals. For each animal the percentage difference between the two regions has been given. For further details see text.

Animal	Large islets I. Peripheral	Large islets II. Central	Difference I-II
8	25.7	19.0	+6.7
9	17.3	17.6	—0.3
10	12.9	17.1	—4.2
11	18.0	10.5	+7.5
12	8.1	7.7	+0.4
13	6.5	7.0	—0.5
14	17.6	15.0	+2.6

DISCUSSION

It is usually accepted that an incorporation of tritiated thymidine in a nucleus is coincident with the duplication of DNA and reflects synthesis of new chromosome material.[5-9] By combining autoradiographic and photometric measurements on the same population of cells it has recently been shown,[9] that there is no reason to postulate a special "metabolic" DNA, as has been suggested by Pelc.[10-12] The stability of DNA in the cell nucleus except during cell division means, that, after administration of thymidine, the radioactivity should be found in cells forming DNA in preparation for division at the time of injection, and once labeled cells should remain so for long periods of time depending on the interval before the labeled cells again divide. After several such divisions the remaining radioactivity is insufficient to produce an autoradiographic image, i.e., the frequency of labeled cells decreases. Thus, under certain predictions the extent of decrease can be said to reflect the renewal of a cell population, a fact that has been especially pointed out by Leblond et al.[5] For the type of experiment where young animals are injected with ^{3}H-thymidine and the frequency of labeled cells studied after intervals up to several months, the latter authors introduced the term "cell retention test."

A not inextensive cell renewal must constitute a necessary requirement for the considerable postnatal growth, which has been previously observed in the rat as regards both the endo- and exocrine parts of the pancreas.[1-4] However, since the net growth of a tissue is also influenced by the extent of cell destruction, it has been possible to provide further information on the growth pattern in the islets and the exocrine parenchyma from autoradiographic studies of the DNA metabolism in these tissues carried out by the "cell retention test." The fact that the curve, which in our experiment represents the percentage number of labeled cells at different ages, shows a more gradual fall in the case of the islets, may best be explained as an expression of the fact that the relatively smaller

postnatal increase in volume of this pancreatic tissue is a result of a lower mitotic frequency in the islet cells; *cf.* the "cell retention" curves in figure 3 with the curves for the absolute islet volume and the pancreatic weight respectively at different ages in the rat. A further sign of the parallelism, which seems to exist between the rates of absolute growth and cell renewal, as it appears in the "cell retention test," is that the curves, which represent the percentage number of labeled cells in the exocrine and endocrine parts, tend to reconverge in the older animals. It is typical of the postnatal growth of the islet tissue in the rat, that this is slow at first, but accelerates with increasing age, so that the ratio between the volume of the islet tissue and the rest of the pancreas, after becoming reduced to a minimum value, subsequently rises again significantly in older animals.[1-4] It may be mentioned that the observations of Fitzgerald and Vinijchaikul,[13] that the frequency of labeled cells is at least as high (about 1 per cent) in the islets as in the exocrine parenchyma one to twenty-four hours after an intraperitoneal injection of tritiated thymidine in rats weighing 180 gm. agrees well with the concept that it is only before puberty that the cell renewal is greater in the exocrine tissue.

Since the islet tissue does not constitute a homogenous cell population, but is composed of at least two cell types with different functions, it would be of interest to compare also the frequency of labeling in these two types at different ages. A complete analysis of this problem requires the combination of autoradiography with a selective differential staining of the α and β cells. Attempts were made to stain the sections both before and after they were covered with the stripping film. However, no successful results were obtained although we used various methods of granule staining, including the eosin-methyl blue mixture described by von Mann, which has been found especially suitable for rat islets. In those groups of α cells, which because of the characteristic nucleo-cytoplasmic volume ratio could be easily identified in the periphery of the islets in the older animals, both the frequency of labeled cells and their degree of blackening appeared to be higher than in the rest of the islets. The fact that even so it was not possible to demonstrate any higher frequency of labeling in the periphery of the large islets than in their central regions is probably due to the comparatively large random error in these particular determinations. In the case of the β cells, we could also discern a possible tendency to a larger percentage frequency of labeling in the islet centers. If the existence of differences in the distribution of the radioactivity between centrally and peripherally situated β cells could, in the future, be confirmed on sections with an adequate differential staining, this would be an important contribution to the discussion on whether there are special zones for growth and decay within the islets (*cf.* Hughes[14] and Hellerström et al.[15]).

There was a marked variability in the labeling frequency between the animals killed at the same time. This variation may depend upon difficulties in avoiding leakage by intraperitoneal injections in very young rats and in different rates of thymidine absorption from the peritoneal cavity. However, in comparing the islet tissue and the exocrine parenchyma near and far from the islets, it is the differences in any particular rat that are of essential importance. From the percentage figures in table 1 it thus appears that when the percentage labeling is comparatively high in the islets of an animal it is also high in the different regions of the exocrine parenchyma. Even if it is not valid to present the percentage figures as a mean value and its standard error it is easy to recognize, for example, that the statistical probability for getting by chance only negative values for the differences between the exocrine tissue near and far from the islets in the ten animals older than eleven days must be insignificant.

The possibility that the initial greater labeling of the acinar cells near to the islets might be due to inequalities in the thymidine distribution consequent upon the vascular pattern have no support in later studies of the incorporation in vitro of tritiated thymidine in the pancreas of adult mice (Hellman et al., unpublished). The percentage frequency of labeled cells near to the islets in the twenty mice investigated was more than twice the value for the remaining part of the exocrine parenchyma. Neither in these in vitro experiments nor as regards the present studies of the in vivo incorporation of thymidine in rats were there any signs of a more intensive cell proliferation in the regions of the ductules. For the reasons mentioned above, the more rapid fall in the curve, representing the percentage number of labeled cells close to the islets, seems therefore probably a result of the cell division being more frequent in this region than in those cells which lie further away from the islets.

Regional differences in the DNA metabolism of the exocrine tissue can perhaps be explained by the fact that, as a result of the capillary pattern in the pancreas, there is a high local concentration of islet hormone in the vicinity of the islets. Such an interpretation agrees with the hypothesis of Ferner,[16] that in the dis-

semination of the islet tissue there is an underlying functional principle which tends to ensure a strong insulin effect on the exocrine parts of the pancreas. According to Ferner, the particularly high insulin titer close to the islets causes the exocrine cells in these regions to have special characteristics which might account for the long-known halo phenomenon consisting of unusually plentiful and strongly stained zymogen granules in these cells. The idea that the exocrine parenchyma near to the islets is functionally more active is supported by recently made autoradiographic observations[17] that these cells, four hours after an intravenous injection, show a considerably greater uptake of ^{35}S-dl-cystine.

SUMMARY

The net growth of the endo- and exocrine parts of the pancreas has been studied previously in the rat. These investigations have now been complemented by autoradiography of the cell proliferation in the pancreas, as manifested in the DNA metabolism of the cells.

Fourteen rats were injected intraperitoneally on the first and third days of life with ^{3}H-thymidine, and were thereafter killed at different times up to just over five months. The frequency of labeled cells was at first high, but later became reduced rapidly; the greatest relative decrease being found in the exocrine parenchyma. While the initial value for this part of the pancreas was about 55 per cent, on the one hundred and fifty-fourth day of life it was less than 7 per cent. The corresponding figures for the islets were respectively about 45 per cent and 10 per cent. The more gradual fall in the curve, relating the percentage number of labeled islet cells at different ages, may best be explained as an expression for the mitotic frequency before puberty being lower in the endocrine than in the exocrine parenchyma. The tendency of the curves again to converge in the oldest animals is discussed with especial regard to the fact that the islet tissue may be said to show an accelerated growth with increasing age, so that the ratio between the volume of the islet tissue and the rest of the pancreas, after reaching a minimum value, subsequently rises again significantly in older animals.

Characteristic differences were noted between the labeling frequency of the exocrine cells, which were localized close to the islets, and those which were found far from them. The more rapid fall in the curve which represents the percentage number of labeled exocrine cells close to the islets, has been interpreted as probably due to the fact that these cells divide particularly often.

SUMMARIO IN INTERLINGUA

Crescentia Postnatal del Partes Endo- e Exocrin del Pancreas del Ratto: Su Relation con le Metabolismo de Acido Disoxyribonucleic

Le crescentia nette del partes endo- e exocrin del pancreas del ratto ha previemente essite studiate. Nunc iste investigationes ha essite complementate per autoradiographia del proliferation cellular in le pancreas, como illo es manifeste in le metabolismo cellular de acido disoxyribonucleic.

Dece-quatro rattos esseva subjicite a injectiones intraperitonee de ^{3}H-thymidina in le prime e le tertie die del vita. Illos esseva sacrificate subsequentemente a varie tempores de lor vita, con un maximo de justo plus que cinque menses. Le incidentia del marcate cellulas esseva alte al initio. Illo declinava plus tarde rapidemente. Le plus marcate declino esseva trovate, relativemente, in le parenchyma exocrin. Durante que le valor initial pro iste parte del pancreas esseva circa 55 pro cento, le 154te die illo esseva minus que 7 pro cento. Le correspondente cifras pro le insulas esseva, respectivemente, circa 45 e 10 pro cento. Le declino plus gradual in le curva monstrante le relation del procentage de marcate cellulas insular con le etate se explica le melio como expression del facto que le frequentia mitotic ante le pubertate es plus basse in le parenchyma endocrin que in le parenchyma exocrin. Le tendentia convergente del curvas in le plus vetule animales es discutite con referentias special al facto que on pote asserer que le tissu insular monstra un acceleration del crescentia con le avantiamento del etate, de maniera que le proportion de volumine inter le tissu insular e le resto del pancreas —post attinger un valor minimal—monta de novo de maniera significative quando le animales avantia in lor etate.

Differentias characteristic esseva notate in le frequentias del marcation inter le cellulas exocrin in le vicinitate del insulas e le cellulas exocrin a distantia de illos. Le plus rapide declino in le curva pro le procentage de marcate cellulas endocrin de sito vicin al insulas esseva interpretate como effecto del facto que iste cellulas se divide con un particularmente alte frequentia.

ACKNOWLEDGMENT

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On Teaching Diabetes

How Can the Practice of Medicine in the Treatment of Diabetes Be Improved?

Organization in the hospital. It was felt that a diabetes clinic was absolutely essential. There was some debate as to whether this clinic should be a metabolic clinic, an endocrine clinic with a diabetes sideline, or a diabetes clinic. The final consensus was that in smaller institutions it might have to be a mixture, but the larger the institution and the more patients, the more likely it was to be a pure diabetes clinic. That being the case, the function of the diabetes clinic was considered.

Everyone agreed that service is the basic function of the diabetes clinic. But teaching associated with patient care also is a function of the clinic and it was questioned whether good service is compatible with teaching. An internal divison of one of these clinics was suggested whereby a certain section would do undergraduate and graduate teaching and the other section would devote itself almost exclusively to service, with staff being rotated through these sections. Research was believed to have a place in the clinic since the clinic was the source of its material. But it was generally agreed that although the whole diabetes clinic could not be devoted to research, at least a small portion of the clinic should have its efforts focused on research, always associated with service. The relation to other clinics was discussed. Should one have satellites from the diabetes clinic, in the cardiac clinic, in the eye clinic, etc., or should one accept what you might call "Trojan Horses" into the diabetes clinic and have some "circulatory" people and "eye" people there all the time? There was no solution to that; it would all depend on the attitude of the other services.

The discussion of the diabetic inpatient brought forth a debate as to whether one should have a mobile diabetes service and a diabetes ward as well. The diabetes service, most panelists felt, was a sound idea if it was practical in the particular institution. However, a diabetes ward with specially trained nurses aside from the regular medical ward was felt to be impractical from many points of view. The diabetes consultants would be based either in the diabetes clinic or on the diabetes service, and they were regarded as absolutely essential for the good teaching of undergraduates and outside doctors as well. While it was felt that in most institutions it would not be good to have every diabetic seen and treated by a diabetes service, the compromise was to create a diabetes task force, either a single man or a man with a few residents, who would be called in whenever anything became somewhat complicated.

By Peter H. Forsham, M.D., in
Teaching and Research in Diabetes,
Charles C Thomas, Springfield,
Illinois, pp. 24-25, 1960.

Employment Experiences of Juvenile Diabetics

Some Observations Based on a Survey

Abraham H. Kantrow, M.D., Flushing, New York

A questionnaire was sent to 408 juvenile diabetics, alumni of Camp NYDA, the summer camp of the New York Diabetes Association. The objective was to obtain a picture of the educational attainments and employment experiences of these young adults who are now eighteen years of age or older. In addition, an effort was made to delineate some of their clinical and social characteristics. Previous studies of employment of diabetic workers have been based on surveys of the policies of industrial management and the experiences of industrial physicians.[1-4] This survey attempts to investigate the other side of the coin, namely the employment-seeking experiences of the juvenile diabetic — the individual who has grown up with diabetes and who comes to his initial job with diabetes.

It is not known what selective factors were at work in the sample for which data were obtained. Those who wished to share their achievements, as well as those who looked to the New York Diabetes Association as a possible source of help, would be motivated to respond to the questionnaire. Furthermore, no attempt was made to determine the number who have died or the number of those with severe complications who were not motivated to respond. It is not possible to determine if this sample is truly representative of Camp NYDA alumni. Nevertheless, this information is unquestionably useful in bringing out the problems of these young people.

Replies were received from 123 individuals representing 30 per cent of the group. The questionnaires of 116 individuals were sufficiently complete for analysis. The data were divided into three age groupings: eighteen and nineteen, twenty through twenty-four, and twenty-five through thirty-four. This age grouping of the data permits comparison with Census Bureau data and reflects the maturing of the individual through his educational and job experiences.

Presented at the Twentieth Annual Meeting of the American Diabetes Association in Miami Beach on June 11, 1960.

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CLINICAL PROFILE (TABLE 1) RESULTS

The age at diagnosis of diabetes ranges from six months to eighteen years. The peak ages for the males are twelve and thirteen years, and for the females ten and eleven years. The duration of known diabetes ranges from four to thirty years.

All of these juvenile diabetics use insulin. The average dosages decrease for each successive age group for both males and females. The average dosage of the females is less than that of the males for all groups. Although these data are not based on longitudinal studies they appear to bear out the observation that there is a diminishing need for insulin after the adolescent becomes fully mature. Insulin dosages range widely from ten units to 160 units. Three individuals take less than twenty units.

Six individuals take oral hypoglycemic medication in addition to insulin.

The question regarding personal rating of diabetes control was asked to obtain some impression of how these young people feel about themselves. Are they, by and large, satisfied with the management and control they exercise over their condition? Whether they are justified or not, the majority express satisfaction with their diabetes control. Seventy-one, 61.2 per cent, rate their control as "good," forty-one, 35.3 per cent, as "fair," and four, 3.5 per cent as "poor."

Two individuals, a male aged twenty-six and a female aged twenty-nine, report that they are blind. The female was a personnel supervisor with a major radio network before her vision became impaired. One male, aged twenty, reports the onset of cataracts. He is an electric fork-lift truck driver. Failing vision increases the hazards of his present job and makes him apprehensive about seeking new employment. His answers to the questionnaire reflect his desperation and his need for medical supervision and vocational guidance.

TABLE 1

Clinical profile

Age group	18-19		20-24		25-34		Totals
Sex	M	F	M	F	M	F	
Number	17	16	25	21	20	17	116
Age at diagnosis							
Average	8.4	9.7	8.3	7.7	10.3	8.8	
Range	3-13	7-14	½-13	4-13	2-18	4-14	
Duration of known diabetes (years)							
Average	10.0	9.0	13.8	12.0	17.9	10.5	
Range	5-16	4-12	8-22	7-20	10-30	15-26	
Insulin dose units							
Average	76.7	59.6	66.0	49.3	55.9	43.2	
Range	50-105	15-100	25-115	20-82	10-160	15-80	
Insulin and oral hypoglycemic compound	2	2	2	—	—	—	6
Personal rating of control							
Good	9	9	14	12	12	15	71
Fair	7	7	5	4	13	5	41
Poor	1	—	1	1	—	1	4
Complications							
Blind	—	—	—	—	1	1	2
Cataracts	—	—	1	—	—	—	1

EDUCATIONAL ATTAINMENTS (TABLE 2)

Educational attainments range widely from the minimum of grade school to the acquisition of advanced degrees in the arts and sciences.

Two males have no formal education beyond elementary school. One hundred and nine of the 114 who attended high school have graduated. This represents a graduation rate of 95 per cent and stands in sharp contrast to the graduation rate of 55.76 per cent for all high school students in New York City.[5] However, it should be noted that this high graduation rate may not be typical of the total Camp NYDA alumni group. The motivations which elicited replies to the questionnaire may have weighted the results in favor of response from those with higher educational attainments.

Sixty-six of the high school graduates have gone ahead to college. Twelve have left college before graduation. Thirty-three are attending college now. Nineteen of these thirty-three are full-time students and fourteen have full-time employment and attend college at night. Twenty-one have college degrees and nine have gone

TABLE 2

Educational attainment

Age group	18-19		20-24		25-34		
Sex	M	F	M	F	M	F	Totals
Number	17	16	25	21	20	17	116
Completed grade school	17	16	25	21	20	17	116
Partial high school	4	1	—	—	—	—	5
Completed high school	12	15	24	21	20	17	109
Partial college	—	2	2	2	4	2	12
College student	8	5	10	6	4	—	33
Completed college	—	—	6	5	6	4	21
Postgraduate student	—	—	3	2	3	1	9
Postgraduate degree	—	—	—	—	2	2	4
Vocational training	1	5	5	7	9	3	30

ahead to graduate work. Four have their master's degree and two are working for their doctorate. Thirty of the group have had, or are having, some type of vocational training, either as part of secondary school education, or in addition to high school and college education.

MARITAL AND FAMILY STATUS (TABLE 3)

The marriage rate for this group of juvenile diabetics is considerably lower than that reported by the Bureau of Census for the urban population of the United States.[6] This is true for both sexes and for each age group. Eight of the thirteen married males have fourteen living children, and seven of the twenty-one married females have ten living children. Two females have experienced five miscarriages. Neither of these females has living children. Four females and the wife of one diabetic male are currently pregnant for the first time.

PRESENT OCCUPATIONAL STATUS (TABLE 4)

Occupations range from responsible positions in a number of professional areas to unskilled labor. The greater number of individuals, thirty-six or 31 per cent, fall into the category of office workers. Nineteen full-time students represent 16.3 per cent of the group. Seventeen, or 14.6 per cent, are currently engaged in professional careers. Among the males there are nine accountants, three engineers, one economist and one social worker. Among the females there is one of each of the following: librarian, occupational therapist, and teacher. The single practicing teacher is a member of a religious

TABLE 3

Marital and family status

Age group	18-19		20-24		25-34		Totals	
Sex	M	F	M	F	M	F	M	F
Number	17	16	25	21	20	17	62	54
Marriage rate								
Number	—	3	2	7	11	11	13	21
Per cent	—	18.7	8.0	33.3	55.0	64.7	—	—
Census Bureau rate	9.7	31.5	48.2	65.6	77.9	83.6	—	—
Living children								
Number with children	—	—	—	1	8	6	8	7
Number of children	—	—	—	1	14	9	14	10
Miscarriages								
Females with loss	—	—	—	1	—	1	—	2
Number lost	—	—	—	4	—	1	—	5
Current pregnancies	—	2	1*	2	—	—	1*	4

*Wife of diabetic

TABLE 4

Present occupational status

Age group	18-19		20-24		25-34		Totals	Per
Sex	M	F	M	F	M	F		cent
Number	17	16	25	21	20	17	116	100.0
Office	1	6	4	13	2	10	36	31.0
Student	7	4	5	3	—	—	19	16.3
Professional	—	—	6	2	8	1	17	14.6
Skilled	3	—	7	—	7	—	17	14.6
Housewife	—	2	—	2	—	5	9	7.7
Unemployed	2	3	1	1	1	1	9	7.7
Sales	2	1	2	—	—	—	5	4.5
Unskilled	1	—	—	—	1	—	2	1.8
Self-employed	—	—	—	—	1	—	1	0.9
Reformatory	1	—	—	—	—	—	1	0.9

teaching order and is combining her teaching with graduate work towards a master's degree in education. One other young woman is a member of a religious teaching order, but she is a full-time student at a normal school and is classified as a student. Two housewives are former teachers who are now raising families and are classed as housewives.

Skilled workers number seventeen and comprise 14.6 per cent of the group. The types of skilled craft represented by the group include carpentry, steam fitting, graphic designing, printing and photography, electronic and X-ray technology, machine tooling, business machine operation, telephone transmission, and fork-lift truck operation.

Only nine of the twenty-one married females classify themselves as full-time housewives.

Nine individuals are currently unemployed. Five are eighteen and nineteen years of age and are on the threshold of their employment experience. The two eighteen-year-old males have worked as unskilled laborers and the three females have not, as yet, had their first employment experience. Two in the oldest age group are blind and unemployable. One male and one female in the twenty through twenty-four age group are currently unemployed. The male, aged twenty-three, has no special skills. The female, aged twenty-two, has a college degree in education and child psychology. She had been rejected by the New York City Board of Education for a teaching position and her comments on the questionnaire reveal her plight in planning for a career and in seeking employment:

> The Board of Education here in the city has refused to give me a license on medical grounds. Because the field did not appear to be closed to diabetics, I was educated for the teaching profession. However, much to my heartache, I find it closed to me here in the city and in areas outside the city. Because I felt that my diabetes should not be a reason for my not obtaining a position I have been completely truthful on my application forms. However, at this time, I feel that my lying on the application blanks might be the only solution.

A number of respondents, both male and female, comment about Board of Education restrictions which barred them from teaching careers. Fortunately these restrictions no longer operate in New York City.*

The remaining occupational categories, sales, unskilled, self-employed and a single reformatory inmate, comprise 8.1 per cent of the group.

*During the past year the New York City Board of Education has lifted its restrictions on the employment of the well-controlled diabetic teacher. This change of policy followed a series of conferences with representatives of the New York Diabetes Association.

PROBLEMS EXPERIENCED AT WORK

Seventeen individuals, 14.6 per cent, indicated that diabetes has presented some problem in their work experience. The group, fifteen males and two females, comprises seven skilled workers, three unskilled workers, three office workers, two graduate students, one professional and one housewife.

All individuals relate their problem to symptoms of hypoglycemia which, with one exception, are easily controlled. The single exception is a male who has had "hundreds" of insulin reactions and on several occasions has required intravenous glucose. His work capacity and employability have suffered considerably. In addition to symptoms of hypoglycemia, three males observe that they occasionally experience symptoms related to hyperglycemia. In five instances the problem of diabetic stability is thought to be related to irregular work shifts and irregular heavy work loads. One individual, a fork-lift truck operator, as mentioned above, is handicapped in his work by the onset of cataracts.

Two young men in this group take 130 and 160 units of insulin daily. The average insulin dosage of the remainder of the group does not deviate from the averages of the entire group.

INCOME (TABLE 5)

Seventy-six respondents noted their salaries. These figures are compared with the Census Bureau analysis of income in the northeastern section of the United States.[7] It is apparent that the average income of these diabetics compares favorably with the figures provided by the Census Bureau.

EMPLOYMENT EXPERIENCES (TABLE 6)

The employment experiences of seventy-seven full-time workers are analyzed in table 6.

Forty-one of the seventy-seven full-time workers, 53.2 per cent, have been refused employment because of diabetes. No particular occupational category stands out with regard to job refusals. Approximately one half of the individuals in all the listed categories of work have had this experience.

A number of large corporations including manufacturing, telephone, public utilities, department stores, and a railroad are specifically named by the respondents as having rejected their application for employment because of diabetes. In addition many individuals were rejected by banks, brokerage houses, insurance companies, libraries, civil service systems, and boards of education.

TABLE 5

Income of seventy-six respondents

Income	Diabetic males No.	Diabetic males Per cent	Percentage male Northeast* U.S.A.	Diabetic Females No.	Diabetic Females Per cent	Percentage Female Northeast* U.S.A.
Less than $3,000	11	25.0	39.3	8	25.0	83.3
$3,000 to $5,000	18	40.9	39.4	22	68.7	14.3
$5,000 to $10,000	13	29.5	18.4	2	6.2	2.2
$10,000 and over	2	4.5	2.7	0	—	0.4
Totals	44	99.9	99.8	32	99.9	100.2

*U. S. Bureau of Census.[7]

TABLE 6

Employment experiences of seventy-seven full-time workers

Occupation	No.	Experienced job rejection	Never experienced job rejection	Experienced job rejection: Acknowledge diabetes	Experienced job rejection: Now conceal diabetes	Never experienced job rejection: Acknowledge diabetes	Never experienced job rejection: Always conceal diabetes
Office	36	19	17	10	9	7	10
Professional	17	9	8	7	2	6	2
Skilled	17	10	7	4	6	5	2
Sales	5	2	3	2	—	1	2
Unskilled	2	1	1	—	1	1	—
Totals	77	41	36	23	18	20	16
Percentages	100	53.2	46.6	29.8	23.4	25.9	20.6

There is, apparently, no consistent policy regarding the employment of juvenile diabetics. Large and small concerns, private companies and civil service systems alike have employed them and refused them employment. Some department stores employ diabetics, others do not. Some insurance companies will employ them, others will not. Similar conditions exist among accountancy, banking and engineering companies.

Eighteen of the forty-one who have been rejected for employment now conceal their diabetes when applying for work. Many respondents describe bitter experiences which compel them to deny their diabetes in order to gain employment. Sixteen of the thirty-six who have never been rejected for employment because of diabetes have always concealed their condition. Combining these two groups, thirty-four of the seventy-seven full-time workers, 44 per cent, currently conceal their diabetes when applying for work. Many respondents describe the subterfuges they use to obtain employment. False answers have been given to questions regarding diabetes and military draft status. Substitute urine specimens have been submitted at medical examinations.

It is of interest to note that the practice of concealment is much less prevalent among the professional workers. Diabetes is denied by only four of the seventeen professional workers, 23.4 per cent, in contrast to nineteen of the thirty-six office workers, 52.7 per cent, and eight of the seventeen unskilled workers, 47.0 per cent.

Forty-three individuals, 56 per cent of the group of full-time workers, have always acknowledged their diabetes when seeking employment. Twenty-three of these individuals have been refused employment because of diabetes.

DISCUSSION

Diabetes has apparently not prevented those adolescents and young adults who responded to the question-

naire from achieving an educational level which compares favorably with the general population. Vocational placements range over wide areas from the unskilled to the skilled and the professional. Income levels are related to occupations and educational achievements and reflect the ability of the juvenile diabetic to carry out the responsibilities of his work successfully.

The adult-onset diabetic has had the opportunity to prove himself as a worker before the onset of his diabetes and need anticipate little or no change in his employment status when his diabetes is diagnosed. On the other hand, the juvenile diabetic comes to his first job with diabetes and has to demonstrate his value as a worker. Because of diabetes many of these young adults are summarily rejected for employment. Bitter job hunting experiences are cited frequently by the respondents to the questionnaire. When faced with the responsibility for earning a livelihood, the barriers to employment force many who responded to the questionnaire to resort to deliberate falsification and deception. They may conceal their diabetes on application forms, resort to deception regarding their draft status, and attempt to conceal their condition at physical examinations. One can with justification lay the blame for this situation upon the archaic employment policies which still prevail among many large and small commercial and industrial firms. The obstacles to the employment of the well-controlled juvenile diabetic call for a broad educational program designed to change unenlightened employment practices wherever they may exist. The low absentee record of the well-controlled diabetic and his inclusion in all forms of group insurance without jeopardy to the employer need to be widely publicized among personnel workers, industrial physicians and labor unions.

As a result of this survey the New York Diabetes Association has established a Vocational and Counselling Service to help adolescents and young adults in the areas of social and emotional adjustment, education and career planning, and employment. It is felt that the service of such individuals will prove to be valuable to the young diabetic, his family, his physician and the community.

SUMMARY

A questionnaire was addressed to 408 juvenile diabetics (alumni of Camp NYDA) who are now eighteen to thirty-four years of age. Information on diabetic history, educational achievement, marital status, and employment experience was obtained from 116 individuals.

The majority of the respondents are functioning well in a wide variety of situations. Educational attainments are above average. Vocational placement and income reflect ability to work. However, job seeking experiences reveal the hazards and uncertainties faced by the juvenile diabetic. The findings indicate that a broad program of guidance for the adolescent diabetic, and a reorientation of employment practices are needed.

SUMMARIO IN INTERLINGUA

Experientias Occupational de Diabeticos Juvenil: Observationes Basate Super le Resultatos de un Enquete

Un questionario esseva adressate a 408 diabeticos juvenil (alumnos del Campo NYDA) de etates de inter dece-octo e trenta-quatro annos. Informationes in re le historias de diabete, le attingimentos educational, le stato marital, e le experientias occupational esseva obtenite ab 116 del 408 subjectos. Le majoritate del respondentes functiona ben in un grande varietate de situationes. Le attingimentos educational esseva plus que le media. Le experientias in le effortio de trovar un empleo revela le hasardos e le incertitudes con que le diabetico juvenil se trova confrontate. Le constatationes indica que un comprehensive programma de consilio pro le diabetico adolescente e un re-orientation del practicas de empleo es requirite.

ACKNOWLEDGMENT

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Remembrances of 1921

Charles H. Best, M.D., D.Sc., F.R.S., Toronto

FREDERICK G. BANTING

CHARLES H. BEST

The invitation to write an editorial for *Médicine et Hygiène* to be published during the Congress of The International Diabetes Federation, has given me great pleasure. This year the Fortieth Anniversary of the Discovery of Insulin is being celebrated in several places. The occasion brings back, of course, many memories of the exciting days which Fred Banting and I shared during the summer of 1921. I recall very vividly those strenuous weeks when we experienced periods of disappointment, but later, also of elation. Investigators are all too seldom privileged to feel this latter emotion during a lifetime of medical research. Possibly it was a good thing that Banting and I were completely undisturbed during the first four months of our work together. The hot summer days were packed with uncertainty, excitement, pleasure and, above all, activity. As we had no helpers we shared all the usual laboratory tasks which included washing and sterilizing equipment, care of animals and cages, etc. The first attempts to produce a diabetic condition upon which we wished to study the effect of a pancreatic extract, were not successful. The operating facilities available in the Medical Building left much to be desired and we could not achieve the necessary degree of asepsis. We finally abandoned the Hédon procedure (a two-stage technic of removing the pancreas) in favor of the now well-known one which accomplished complete pancreatectomy at one operation. Our trouble was that infection occurred after each surgical procedure and we found it easier to secure satisfactory diabetic animals after one than after two operations. We worked as hard as we could in an effort to show that a neutral or preferably an acid aqueous or alcoholic extract of degenerated or intact dog pancreas and of fetal or adult beef pancreas always provided a potent hypoglycemic material. We repeated and repeated our observations until, on seventy-five consecutive occasions without failures, we were able to secure an extract which successfully lowered the blood sugar and produced a dramatic improvement in our depancreatized animals. We had, of course, a great many advantages over those

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who had searched before us for the hypoglycemic hormone. Undoubtedly one of the most important was Banting's expert surgical technic. Another was the excellent method at our disposal for estimating sugar in small amounts of blood. We were guided hour by hour by the depth of color of our beacon—the tubes containing our blood sugar reagents.

We often slept in the laboratory because we wished to make observations twenty-four hours a day. We would cook our sketchy meals over the Bunsen burner and talk and talk. We had ample opportunity to discuss many things of vital importance to us both. Naturally the topic which fascinated us most was what the future would hold when unlimited amounts of the material which we called "isletin" and later "insulin" would be available. This opened up such vistas that it was difficult to control our excitement. These talks strengthened our determination to push ahead with our experiments which we fully realized held promise for diabetics the world over. Even those who have never engaged in medical research will have no difficulty in understanding what tremendous satisfaction Fred Banting and I had when our diabetic animals responded to the administration of insulin. It is hard for one to describe the elation and stimulation of such an event. Many investigators know from their personal experiences what this means. Our funds were low—neither of us received any stipend during that summer—but the difficulties in both our living and working arrangements only served to add fuel to a fire which burns brightly in all ambitious young men. This period was certainly one of the high points of Fred Banting's life, as it certainly was of mine. During the past forty years interest in insulin has been maintained to such an extent that since our first paper some 80,000 communications on insulin have appeared. Thus we see that all the problems have not been solved and that the incentive is as strong as ever to learn more about insulin and to improve in every possible way the treatment of diabetics throughout the world.

Toronto, May 29, 1961.

I Have Lived for Forty Years the Life of a Diabetic Patient*

R. D. Lawrence, M.D., F.R.C.P., London

Doctors often specialize in diseases which they have themselves. It is common for the directors of sanatoria to have, or have had, tuberculosis. I certainly took up diabetes because it took me up; it was discovered in an unusual way.

After the 1914-1918 war I was doing a House-Surgeon's job in the Ear, Nose and Throat Department of King's College Hospital, and studying to finish the FRCS England. Before the days of sulpha drugs and antibiotics there were many deaths from mastoid disease and its intracranial complications. It was my habit to investigate the exact condition in those who died and then practice the operation on the other side. Some might say "Oh, shame," and others praise my method of improving my knowledge and skill. One night a chip of bone flew into my right eye, which went violently septic. This spread to the other eye and things went from bad to worse in spite of local treatment and two operations under anesthesia.

It was usual where I was warded for the Night-Sister to teach the probationers how to test urine, which was done in the side room of the ward; and one night they happened to test mine and found it loaded with sugar. Our biochemist, Dr. G. A. Harrison, found my blood-sugar over twice the normal and diagnosed diabetes without doubt. This had never been suspected as I had been looked upon as a healthy and vigorous young man indeed, playing tennis and hockey with no classical symptoms of diabetes—no noticeable thirst nor polyuria; and the inclination to fall asleep studying in the late evening is, and I think you will agree, quite physiological and normal in the study of anatomy and other dull subjects.

I was starved and put on the Allen treatment which soon reduced the sugar to traces and the eye sepsis rapidly subsided, although it left the vision of one eye permanently damaged.

After this I established a reasonable carbohydrate tolerance —about 150 gm. A surgical career was now impossible and I turned to medicine, but whenever I worked hard my blood-sugar rose and my tolerance dropped.

By now I realized that my prognosis for health or even long life was very bad and as I did not want to die at home, full of an anxiety situation nor to be longer a financial burden to my good and still willing father, I decided to look for a quiet general practice which would provide (with luck) a modest subsistence. So with the advice and helpful introductions from my senior surgeon I set up in Florence, Italy, to do general practice among the English-speaking residents and travelers often upset from unusual food and wine. I was fortunate in getting a consulting room in the main street, and soon I had

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Reprinted with permission from Médecine et Hygiène, Journal du 4e Congrès Fédération internationale du diabète, *July 10 and July 11, 1961.* Dr. Lawrence is Consulting Physician, formerly in-charge, Diabetic Department, King's College Hospital, London, S.E. 5.

* This is the title I was given and so the writing is bound to be egotistical but I hope not annoyingly so.

enough patients to support myself and, in their absence, to enjoy the glorious architecture and picture galleries for a few months in reasonable health and enough energy for some tennis and dancing with an attractive Dutch sculptress. Then bronchitis made my diabetes too bad (I was loaded with sugar and acetone) for these pleasures and I could hardly keep awake even to interview or visit a new patient. I cut my diet, especially the carbohydrate, to the minimum possible; breakfast, no bread or rolls; cheese, lettuce, celery, olives, black coffee. The meat and vegetable meals at lunch and dinner were tolerable, especially when a glass of dry Chianti wine was added; this gave a little energy, as the French physicians of the eighteenth century had found when they prescribed a liter a day of vin ordinaire. In spite of this rigid regime I had constantly heavy glycosuria (3 to 4 per cent) and a dangerous amount of ketones. All my energy and muscles disappeared. I could not walk up the stairs to my pension room and would even fall down. It was fortunate there was a lift, so I struggled on.

Dr. Harrison wrote from London that there was a new substance, called insulin, reported from Canada, which sounded promising. But I had had so many disappointments by trying quack remedies that I wrote back without enthusiasm saying I would wait and see, without feeling any optimism. When peripheral neuritis was added to the other troubles, so that I could not handle a match to light the solacing cigarette, I felt that the struggle to keep alive—it could not be called living—was no longer tolerable nor worth while, although Florence had some beautiful sedentary compensations in architecture, pictures and music. Then just in time came a cable from my trusted Dr. Harrison.—I've got some insulin, it works, come back quick. We both knew coma was approaching.

Two letters to Dr. G. A. Harrison after he had cabled about having insulin

Florence, Italy
10th May, 1923

My dear Harrison,

I am going to try to get home and have a fortnight with you before you go on holiday. I hope you can find me a bed somewhere there: perhaps you have now got a bit of a ward for our diabetics. I don't seem able to free myself of sugar (+++) on a very small diet and have now got distinct numbness of fingers and feet.

16 May
Florence

Dear G. A. Harrison,

I am leaving here soon and shall turn up on Monday, 28th. May, starving before breakfast. I am looking forward to the treatment very much and have assumed that I shall be benefitted, so shall be very bad tempered if it doesn't work. I don't like peripheral neuritis. It interferes with work.

Cheerio. Till then,
Yours,
R.D.L.

So, I left my growing practice, bundled into my Fiat, with an Italian co-driver who wanted to visit his relatives in Soho, London. The journey was pretty tough going for me as my chauffeur was frightened of the Paris traffic and the English left-side of the road, but I landed up into a borrowed bed in the Casualty ward of King's College Hospital, more dead than alive, but no precoma, and the profound sleep was more from travel exhaustion than ketosis. I had no insulin that night as my previous state was to be studied so as to make me a good guinea-pig for the clinical effect of the new drug.

Next morning I reached the laboratory as soon as it was opened by Miss Taylor, technician, and was found to have a fasting blood-sugar four times the normal, 410 mg. per 100 ml., and a urine loaded with ketones and sugar. When Dr. G.A.H. arrived he at once opened the insulin "frig" and after some discussion in our mutual ignorance, it was decided to give me 20 units (10.00 a.m.) which he did so skilfully in my upper arm that I thought nothing of it compared with some horrid injections I had had of anti-tetanus, typhoid, cholera—so I had no qualms about future insulin injections. Then a real breakfast, bacon and egg perfectly cooked over Bunsens', and actually 30 gm. of bread with no feeling of guilt. Hourly urine tests showed decreasing sugar and acetone until at 3.00 p.m. the urine was sugar-free, and although I felt no better, this led to a cheer for insulin and Banting and Best. In another hour I felt weak, sweaty, with an intense hunger, which led me to the biscuit box and slow restoration, obviously my first hypoglycemic attack. In a few hours glycosuria recurred. We learned in a few days that two injections (15/13 for me) were necessary to keep the diabetes reasonably at bay.

During the first week of insulin I gained little energy and still found it a struggle with aching legs to walk up the stairs to the laboratory but I did gain an encouraging 10 lb. in spite of a still rather low preinsulin diet—75 gm. C., and 1150 calories. After this I made the mistake of following a suggested idea, that by keeping the blood-sugar at a low normal figure, the islets might recover or at least improve their insulin function. So I spent a weak and miserable two months on only 20 gm. C., and enough insulin (8/6 units) to cause daily hypoglycemia and as low a blood-sugar as is humanly tolerable. At the end of this experiment my insulin requirements were the same as in the first week. Another publication suggested that raw pancreas by mouth was effective instead of insulin injections. This was the worst experiment I ever tried on myself; to chew, swallow and keep down raw pancreas—no "sweetbreads" in this form—was a terrible and nauseating hardship, and after swallowing it felt as if the gullet were being digested. A serious return of sugar and acetone occurred with disproof of this nonsense. Then a return was made to a reasonable carbohydrate regime and insulin and to normal working health.

There was still only one type of insulin, now called "regular" or soluble. Some 100-150 gm. C. was given mostly after the two injections—before breakfast and the evening meal —with a little C. 3-4 hours after injections to "buffer" and prevent times of hypoglycemia. By now I had been made biochemist as Dr. Harrison had left for another job, and the regular hours and exercise made diabetic control easy. I began to play games, tennis and squash, and found 5-6 p.m. the safest time, as the morning insulin was nearly finished and violent exercise produced no hypoglycemia, but I learned that I had to reduce the evening insulin a little to avoid hypo-

glycemia at night, or perhaps better (more physiological) to have three sandwiches before midnight.

My first ski-ing holiday required skilful adjustments, both for climbing up (few ski-lifts then) and for getting up from falling down into deep snow—equally strenuous. On ski-ing days I reduced the morning insulin from 15 to 10 units, had an extra roll for breakfast and filled two rolls with honey or black cherry jam to eat after climbing; I also took the usual packed lunch and, of course, sugar in every pocket. On return to the hotel after this very strenuous day, I often felt not only exhausted but very hungry and slightly thirsty, obviously getting diabetic with the reduced morning dose quite exhausted; so I often took an extra small injection (6-10 units) before a fairly normal tea—coffee usually, with a few sandwiches and a cream cake or two—bathed and slept, and took a much reduced dose before dinner, as some of the tea-time dose was still active: and so, with this and other slight modifications I became as fit and strenuous as anybody. My acquaintances would not believe, and I could hardly believe it myself, that I was a severe diabetic nearly dead some months earlier. Only the injection and the balance of insulin by diet to remember, and the effect of exercise as the other important influence, apart from nervous strain and tension increasing sugar—of which there was none in Switzerland.

When ski-ing conditions were bad I took to the skating rink, much easier because one could rest in the sun and lunch appropriately in the hotel, but I was silly enough to try some figures far too difficult for my capacity and fractured my right elbow in a backward fall, and the long splint made self-injection difficult: but for the journey home the doctor shortened the splint, making my own few injections possible on the way back to London to take up the general laboratory routine again, but I was mainly concerned with the treatment of the growing number of diabetics living on insulin.

I discovered that my own routine exercise had a prolonged as well as an immediate effect: for instance, after a strenuous week-end I had to reduce my insulin dosage on Monday and Tuesday, but by Thursday I returned to my usual sedentary dose. In the treatment of my invaluable diabetic laboratory assistant (H. R. Millar) and myself we noticed clinically important variations in batches of insulin and for a time before the more accurate new "cross rabbit" test was introduced we actually tested new batches on ourselves before they were issued for public use. I had only one serious misadventure in all these testings. British insulin was terribly expensive in the early days of its production, so when an imported German insulin was offered at one third the price I tried it on myself and the hospital patients. One Sunday I took the usual dose, I thought, and set off driving to the country. In three and one-half hours I saw double and felt confused and very overdosed, was rescued by all the sugar I had and then took an early lunch, after which I thought myself safe, but the symptoms recurred in another hour and it was fortunate that a sweet-shop was nearby. When I reported this to the M.R.C. (Medical Research Council) their animal test showed the insulin to be twice as strong as the supposed standard. Their further investigation showed this insulin to be prepared in a primitive lab, in a shed behind an abattoir. This was the end of our use of German and unknown insulins, but the British insulin soon came down in price until it became probably the cheapest and the best in the world.

I was soon asked to lecture to the local doctors' society on Diabetes, Diets and the New Insulin Treatment. It was obvious that busy practitioners would have no time nor knowledge to teach details to their diabetics by existing schemes of diet; so I was fortunate to invent a simple method—the Line-Ration, which has been in popular use—to supply accurate weighed diets. This became the basis of the small practical book I was urged to write about the new treatment and which I called the *Diabetic Life*. In preparing its food tables I became aware that the C. composition of foods given by various world authorities varied by over 100 per cent—not good enough for insulin balance—and so I thought it necessary to retest the available C. of all common foods. This was far beyond my chemical capability and it was fortunate that an expert chemist (R. A. McCance) came from Cambridge to study medicine at King's College Hospital and was able to carry on the analyses which were ultimately published as a report by the M.R.C., the "Chemical Composition of Foods."

Now I shall be increasingly egotistical and write more about what I did than about my own life as a diabetic patient; the latter was regularised in an easy routine. I lived in our Students' Hostel five minutes away and, indeed, was the Warden-in-charge. Here my life was easy and suitably regular: morning insulin 7:30 a.m., units 20+; breakfast 8 a.m. 40 gm. C.—bread, marmalade, milk in coffee, bacon and egg or fish, according to the students' breakfast. Five minute walk to the hospital laboratory. At 11.00 a.m., 20 gm. C. to "buffer" any possible hypoglycemia before lunch; 30 gm. C. at 1:30 p.m. (13:30) from the Diet Kitchen; evening insulin 16 units+ at 6:30 p.m. (18:30); dinner 7 p.m. 40 gm. C. Bed-time 20 C., or 40 C. if urine test blue, to prevent hypoglycemia at night. No time wasted on travelling so plenty time for routine work and for experiments with insulin and other drugs, of blood-sugars mostly on Millar and myself but also others under our treatment; and these same drugs on the glycogen in animals, mostly rats. Also the nearness of the hospital enabled me to go at night to observe and treat personally, coma cases, and so to discover that if they died this was mainly due to dehydration and circulatory collapse, which could be overcome, and not to the failure of insulin to abolish ketosis.

By now, the seats in the laboratory were too few to accommodate the growing numbers sent for diagnosis and assessment of diet alone and/or insulin. Teaching them was impossible there, so a side-room to a ward was set aside and equipped by the generous gift from a rich Australian diabetic. There a special hospital Sister prepared and distributed diets to inpatients anywhere in the hospital, taught a simple diet, insulin injections and urine tests to the outpatients assessed in the lab, and attending for check-ups by the doctors, or sometimes, in simple matters, only by herself. Her devoted work was a great success in maintaining the health and working efficiency of hundreds of patients. But two handicaps to easy and efficient working were obvious and became worse as the number of patients grew and grew.

(1) The overcrowding of the biochemical lab, and the embarrassment of its expanding work by the invasion of diabetics standing everywhere waiting impatiently for tests. It drove a visiting Danish doctor to ask me "How can you work well or even at all under these conditions?"

We moved the diabetic outpatients to a vacated museum,

installed waiting seating for 100 patients, which soon became the average attendance on three days a week. My invaluable diabetic technician came with me and found bench space for the necessary simple chemical tests. So I left the general laboratory to a competent biochemist (which I never was) to the satisfaction of all parties.

(2) The admission of diabetics with serious conditions requiring inpatient treatment depended on the loan of a bed from co-operative physicians who often had insufficient beds for their own patients, so that new bed accommodation was needed for the new treatment. In the days of voluntary hospitals money for expansion had largely to be raised from philanthropic sources; it was estimated that the large sum of at least £20,000 would be necessary for these two purposes. This was not so difficult to raise as was expected. It was a prosperous time in London and many rich diabetics were very grateful for their new insulin treatment, and when the hospital need was explained and twenty told that they would have the honor and pleasure of subscribing £500 each, they responded very well. Mr. H. G. Wells, perhaps feeling hard-up at the time, thought his best help would be a letter of appeal in *The Times*. Enough money was soon collected to begin building a Diabetic Block with sixteen beds and, most important, a coma room ready equipped for such emergencies. Fortunately this was completed before World War II, which it survived intact. The rest of the money came in a legacy from a rich South African who had started his insulin treatment in King's College Hospital. These subscribers formed the nucleus of the Diabetic Association, soon formed to help the growing number of living diabetics.

After a few years of insulin I was restored to full virility and had the prospect of good future health and longevity, and so when I met a lovely and most attractive young woman I felt justified and indeed impelled to persuade her to marry me. We have disproved the gloomy and mistaken fear of parents, whose daughters want to marry long-standing young diabetics, that they will have no family, by having three fine sons.

BOOK REVIEWS

FAMOUS FACES IN DIABETES. *Compiled by Cecil Striker, M.D., with a foreword by Elliott P. Joslin, M.D. $25.00, pp. 250, over 200 illus., Library Edition, G. K. Hall & Co., Boston, 1960.*

Out of the study of diabetes and of the problems following in its wake has grown a vast, complex structure of knowledge. Upon a series of great foundation blocks stands a superstructure built of thousands of individual bricks in an ever-growing pattern tracing the design of the metabolic process. To comprehend it fully demands more than familiarity with the two-dimensional present; the third dimension of its evolutionary perspective must be added.

To compile a detailed record of the development of this immensely important branch of science would be a memorable and monumental task. Cecil Striker, who has been so close to many centers of activity in diabetes in recent years, has sensed the importance of such a record and has been willing to take on the labor of drafting its outline. His approach has been by way of a biography of the personalities who have made its history, a "Who's Who" of diabetes, with a portrait and thumbnail sketch of each man and his contribution. Even though many are too brief to satisfy one's curiosity and interest, and despite inevitable omissions—chiefly of contributors to the current explosive expansion of research—it is remarkable that a single author has been able to give us so complete a bird's-eye picture of the subject within the limits of these brief sketches of 173 personalities.

Since so much of the structure has been built in our own time, it is well that its picture should be drawn for us by one who, as a founder and the first President of the American Diabetes Association, has been so closely acquainted with its contemporary history.

And it is no more than natural that the Association, its outstanding members, and its activities should occupy a large part of the foreground. The history of diabetes is being written here and now, and at a rapid rate. Dr. Striker deserves congratulations and thanks for giving to medical history this picture as seen today.

COMA DIABETICUM. *By Rudolf Baumann, $7.00, pp. 210, Verlag Volk und Gesundheit, Berlin, 1959.*

The author had observed 231 cases of diabetic coma during a relatively short period of five years (1952-57). He is Director of the Diabetes Treatment Center for the city of Berlin to which all cases of diabetic acidosis are sent. Such a centet offers the advantages of both a well-trained team continuously available for emergency treatment, and a teaching service for specialized training. The book comprises mainly a careful analysis of clinical and laboratory findings, therapeutic procedures and results. Most of the statistical data are in agreement with the well-documented observations of other authors. The overall mortality rate was 23.8 per cent. It was highest in patients over fifty years of age, and in patients in whom coma existed twelve hours or more prior to admission to the center. There was a significantly higher incidence in women than in men, (about 3:1), whereas the sex distribution of diabetes in the population was 2:1 (females to males).

Of interest is the author's objection to the generally accepted use of large doses of insulin in the emergency treatment of coma. He employs and recommends small doses intramuscularly or subcutaneously, to be repeated frequently, if necessary. He believes that large doses of insulin, even if free from glucagon, cause an initial transitory aggravation of the hyperglycemia, and moreover may be responsible for "insulin resistance." This belief is based on Pavlov's concept of the "inhibition by supramaximal stimuli." One wonders whether wider use of insulin in larger doses might not have decreased the mortality in this series.

The book is well printed and well illustrated with an introductory chapter summarizing the present knowledge of the pathophysiology of carbohydrate metabolism and of diabetic acidosis.

EDITORIALS

WHY JOIN THE AMERICAN DIABETES ASSOCIATION?

The great increase in the number of medical, public health, and scientific organizations in recent decades, and of publications and meetings devoted to their activities, poses a serious problem to workers in these fields. Which organizations are essential? Which desirable? And which actually superfluous? Selection is not easy, particularly for the person with multiple responsibilities, who has to keep up with current progress in general medicine as well as with one or more of its subspecialties.

The purpose of the American Diabetes Association is not, naturally, to add to this confusion of groups. Rather, it is to decrease it by bringing together into one organization as much as possible of the clinical and research work that has to do with diabetes and with the problems of metabolism related to it.

Diabetes is a prevalent condition, with many complex aspects. Every physician is aware of the damage the illness does to the individual and consequently to his family; its social and economic costs to the community at large; and also, and most importantly, the basic preventability of much of that damage and much of that cost. On the other hand, the tremendous growth in research in biochemistry, physiology, cell structure and function, and pharmacology, and also in basic clinical investigation, have all made increasingly necessary a common meeting ground for those who must keep in touch with the current advances in this subject, and with their fellow-workers in the field.

Or take the social and educational problems which diabetes brings with it. There is the need for social services such as summer camps for juvenile diabetics, where youngsters, particularly those who are city-bound, can have a vacation in the country and also at the same time see how other diabetic children adapt themselves to diet and to insulin injections. There is the never-ending need for widespread and systematic case-finding, with its attendant public education on the nature of diabetes and its symptoms. There is the continuing requirement that the diabetic patient himself learn how to control his condition according to the best and latest methods. And of course there is the urgent need to bring the latest knowledge on the treatment of diabetes to the general practitioner, through whom information on almost all improvements in the management of the condition must be passed to the patient. Each of these difficult and complex problems calls for an organization that is specifically equipped to coordinate resources and energies and disseminate information in this area.

Because of these many and growing demands for organizational, informational, and technical assistance, the ADA finds its programs constantly growing, and its usefulness constantly expanding. To take a single example, the Association's journal, which you are now reading, has become an indispensable instrument of specialized communication between research scientists, professional teachers, and practicing clinicians, through which the ever-increasing amount of information on diabetes and closely allied fields of medicine are disseminated.

On an entirely different level—that of day-to-day activity in treating diabetics and helping them manage their condition—is the Association's program of aiding and promoting the organization of local Affiliate Associations, on a city-wide, county, state, or regional basis. Of the ADA's 2,500 members, many are also members of one of the Affiliates as well. Through these Affiliates, the ADA provides an opportunity for interested laymen to work for the well-being of diabetics and for dissemination of knowledge about the disease and its early symptoms. Each Affiliate has a professional society, which devotes itself to professional education, patient education, case finding and research. Most of the Affiliates also have lay societies as well, through which diabetics and other laymen interested in the disease are able through mixed lay-professional committees to promote programs of public education, case finding, camping, etc. for diabetics.

But the first thing to do—if you have not already done it—is, of course, to join the American Diabetes Association. Write to the Association at 1 East 45th Street, New York 17, N.Y., for an application form for yourself and any of your staff or your colleagues who may be interested. The annual dues include a subscription to this Journal, and are, indeed, one of the main

sources of funds for the support of the work of the Association, which, as an organization, has never made a public appeal for funds.

HENRY E. MARKS, M.D.
New York City

THE FORTIETH ANNIVERSARY OF THE DISCOVERY OF INSULIN

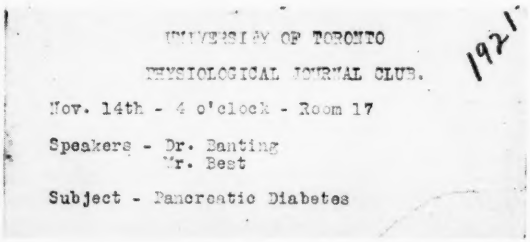
Nov. 14, 1961, marked the 40th Anniversary of the first communication by Banting and Best when they reported the discovery of insulin to the Physiological Journal Club of the University of Toronto on the subject of pancreatic diabetes.* The Journal DIABETES takes note of this occasion with the pride common to all who recall the impact of the achievement heralded by that report.

Frederick Banting's memory is kept fresh by the fact that every day some newly discovered, young diabetic can face life serenely and securely under the protection of insulin. Charles Best, our beloved colleague, moves everywhere as the standard-bearer for good care of diabetic patients, as well as the interpreter of insulin's health-giving properties.

All physicians, investigators and students of medicine have been made richer and better as a result of witnessing or learning the story of the discovery of insulin which has opened unending opportunities for endocrine and metabolic research.

*The first *published* report of their discovery, entitled "The Internal Secretion of the Pancreas," appeared in the *Journal of Laboratory and Clinical Medicine*, Vol. 7, No. 5, February 1922, pp. 251-66.

The memories of Charles Best and the reminiscences of Robin Lawrence (England's great "student-diabetic"), published last summer in Geneva and reprinted elsewhere in this issue, are stirring and valuable recollections of the agony and the ecstasy of 1921-1922 for all


UNIVERSITY OF TORONTO
PHYSIOLOGICAL JOURNAL CLUB.
1921
Nov. 14th - 4 o'clock - Room 17
Speakers - Dr. Banting
Mr. Best
Subject - Pancreatic Diabetes

The simple announcement above appeared on the bulletin board in the Department of Physiology at the University of Toronto preceding the first report by Banting and Best of the discovery of insulin.

diabetics and their troubled physicians. New hope came unexpectedly from modest, unknown sources. The hope became a reality and diabetics ever since have been given countless new lives. The spreading benefits of those saved lives are brilliantly illustrated in another therapeutic miracle when George Minot, an insulin-dependent diabetic, was saved to become a sterling medical scientist and, in time, a Nobel prize-winner as a co-discoverer of the cause and cure of pernicious anemia—another of humanity's medical triumphs. It is, therefore, fitting and pleasant to salute Banting and Best again in this Anniversary Issue.

IRVING GRAEF, M.D.
New York City

ABSTRACTS

Antoniades, Harry N.; Beigelman, Paul M.; Tranquada, Robert B.; and Gundersen, Kare (Protein Foundation Labs., Jamaica Plain, Mass.; Univ. of Southern California, Los Angeles, Calif.; Boston Dispensary and Tufts Univ., Boston, Mass.): STUDIES ON THE STATE OF INSULIN IN BLOOD: "FREE" INSULIN AND INSULIN COMPLEXES IN HUMAN SERA AND THEIR IN VITRO BIOLOGICAL PROPERTIES. Endocrinology 69: 46-54, July 1961.

Insulin is found in the blood in both "free" and "bound" forms. The latter is without activity when assayed by the rat diaphragm method. The ratio of "bound" to "free" insulin in the sera of nondiabetic subjects is quite variable. Separation of the two forms of insulin is accomplished by the exclusive absorption of the "bound" form on a cationic resin. Bound insulin is a basic protein-insulin complex which can be dissociated by the addition of adipose tissue extract. The latter is sensitive both to heat and storage. "Bound" insulin may represent a stored circulating insulin. H.L.W.

Antoniades, Harry N.; Gundersen, Kare; and Pyle, Hugh M. (Protein Foundation Labs., Jamaica Plain, Mass.; and the Boston Dispensary, and the School of Med., Tufts Univ., Boston, Mass.): STUDIES ON THE STATE OF INSULIN IN BLOOD: THE ROLE OF GLUCOSE IN THE IN VIVO DISSOCIATION OF INSULIN COMPLEXES. Endocrinology 69:163-69, July 1961.

Levels of "free" and of "bound" insulin have been determined in both nondiabetic and diabetic individuals before and after the injection of glucose. In the fasting subject insulin is present largely in the "bound" form. Within ten to twenty minutes after intravenous injection of glucose, the levels of "free" insulin rise and those of "bound" insulin fall. The untreated diabetic patient shows a slower than normal rate of

dissociation of "bound" insulin following glucose administration. In spite of a high fasting blood sugar in these patients, the blood insulin is largely bound. The in vitro addition of glucose to bound insulin does not cause its dissociation suggesting that glucose activates a mechanism which in turn promotes the release of "free" insulin. H.L.W.

Barry, Beryl A.; Matthews, J.; and Smyth, D. H. (Dept. of Physiol., Univ. of Sheffield, Sheffield, England): TRANSFER OF GLUCOSE AND FLUID BY DIFFERENT PARTS OF THE SMALL INTESTINE OF THE RAT. J. Physiol. *157*:279-88, July 1961.

The small intestine was removed from anesthetized white rats, everted, and divided into five segments. The ends of the segments were ligated, and the transfer of glucose and water was studied in these sacs in vitro during incubation in Krebs' Henseleit bicarbonate buffer. The capacity for transfer of glucose and water was not the same for different regions of the intestine; the middle section was the most active for both glucose and water transfer. The ileal end was found to have very little capacity for glucose transfer but transferred water at relatively high rates. Reasons are given for believing that water transfer in the jejunum is largely dependent upon the transfer and metabolism of glucose, whereas water transfer in the ileum is glucose-independent. H.T.N.

Bellet, Samuel; Sandberg, Herschel; Tsitouris, George; Muller, Otto; and Schraeder, Jean (Div. of Cardiology, Philadelphia Genl. Hosp., Philadelphia, Pa.): ALTERATIONS IN FIBRINOLYTIC PARAMETERS IN THE HUMAN DURING RECOVERY FROM DIABETIC ACIDOSIS. Metabolism *10*:429-38, June 1961.

The blood of eleven subjects with diabetes mellitus was studied during acidosis and again during recovery from the acidotic state for alterations in fibrinolytic parameters. The following procedures were performed: (1) lysis time of a standard fibrin clot by undiluted and diluted plasma, (2) euglobulin lysis time, (3) plasma fibrinogen concentration, and (4) antithrombolytic lysis times. Normal control bloods were simultaneously studied. The patients in diabetic acidosis displayed more fibrinolytic activity than the normal controls. During recovery from diabetic acidosis this fibrinolytic activity greatly increased. Insulin added in vitro to plasma failed to enhance fibrinolytic activity. This increased thrombolytic activity is attributed to a powerful activator of plasminogen released into the blood stream by the administration of insulin. C.A.R.

Bergström, J.; Findor, J.; and Hultman, E. (Central Clin. Lab., St. Erik's Hosp., Stockholm, Sweden): THE CONTENTS OF GLYCOGEN AND POTASSIUM IN HUMAN LIVER TISSUE OBTAINED BY NEEDLE BIOPSY. Scandinav. J. Clin. & Lab. Invest. *13*:353-54, 1961.

Needle biopsies of liver were performed in six healthy volunteers and in three patients with chronic polyarthritis. One of these had a precirrhotic stage of liver damage histologically. Biopsies were done between 10 and 11 a.m. after the subjects had fasted overnight. A portion of the liver specimen was homogenized with water, and glycogen was determined after hydrochloric acid hydrolysis of a trichloroacetic acid extract, by measuring the glucose liberated. The average glycogen concentration was 4.96 gm./100 gm. wet weight of liver; the precirrhotic liver contained 2.26 gm./100 gm. The potassium content of liver averaged 8.57 mEq./100 gm. wet weight. H.T.N.

Bowdler, A. J.; and Bloom, Arnold: ACROMEGALY, HYPERPARATHYROIDISM AND DIABETES MELLITUS. Proc. Royal Soc. Med. *54*:340-42, April 1961.

A case is reported of a forty-eight-year-old woman with acromegaly, diabetes mellitus, and hyperparathyroidism. The patient noted enlargement of the hands at age thirty-five. She was hospitalized at the age of forty-six for diabetic ketosis. There was no history of endocrine abnormality in other members of her family. Examination in May 1948 revealed prominent facial features and a mild spade-like appearance of the hands; the blood pressure of 150/90 and X rays showed enlargement of the sella turcica, a large mandible, and a suggestion of increased tufting of the terminal phalanges of the fingers. In October 1958 the plasma inorganic phosphorus was 3.6 mg. per cent; in November 1959 the serum calcium was 13, and the inorganic phosphorus 2 mg. per cent. The blood urea was 36 mg. per cent. The daily urine calcium loss was about 750 mg. with a daily calcium absorption from the gut of about 590 mg. Exploration of the neck revealed two enlarged parathyroid glands on the left side and two normal glands on the right. The histologic appearance of the enlarged glands was reported to be consistent with either chief-cell hyperplasia or adenoma. Removal of the two enlarged glands corrected the laboratory evidence of hyperparathyroidism. H.T.N.

Bradley, S. E.; Laragh, J. H.; Wheeler, H. O.; MacDowell, M.; and Oliver, J. (Dept. of Med., Columbia Univ. Coll. of Physicians & Surgeons, New York, N. Y.; and the Renal Res. Unit, CIBA Pharmaceutical Products, Inc., Summit, N. J.): CORRELATION OF STRUCTURE AND FUNCTION IN THE HANDLING OF GLUCOSE BY THE NEPHRONS OF THE CANINE KIDNEY. J. Clin. Invest. *40*:1113-31, July 1961.

A detailed investigation was made of the glucose filtration and reabsorptive activity of the kidneys in three dogs, followed by detailed microdissection and measurements of the glomerular surface areas and tubular volume. The latter two dimensions were considered to reflect the physiological parameters of filtration and reabsorption respectively. There was a good correlation between the anatomical and functional measurements. The findings also indicated that the tendency for reabsorption to be saturated at the same plasma glucose levels is due to a homeostatic structural balance between glomeruli and tubules. S.B.B.

Brandaleone, Harold (New York, N. Y.): EMPLOYABILITY OF THE DIABETIC PATIENT. New York J. Med. *61*:2636-40, Aug. 1, 1961.

The author discusses the employment experience of the diabetic worker, with regard to the responsibilities of both the employer and the employee. The numerous benefits to be derived by management in hiring and retaining diabetic employees are pointed out. C.A.R.

Breithaupt, D. J.; and Leckie, R. B. (200 Bloor St. East, Toronto 5, Canada): DIABETES MELLITUS: A STUDY OF THE PRACTICABILITY OF LIFE INSURANCE. Canad. M.A.J. *85*:299-302, Aug. 5, 1961.

The authors analyze mortality statistics of 1,300 patients

with diabetes mellitus who have been issued life insurance since 1940. There were 126 who died possessing 174 policies. The total death rate was 168 per cent of the normal expected. These occurred with a particular increase of frequency among those with a background of over eleven years of the disease, especially if issued before the age of thirty or after the age of fifty years. Cardiovascular and renal disease, suicide and diabetic coma were the causes of death which were particularly increased in frequency.

The factors which were unfavorable as to longevity were, in increasing order, overweight, the presence of abnormalities in the electrocardiograms and especially albuminuria. Other unfavorable factors included severity of diabetes (in insulin dosage), the greater degrees of glycosuria, and the infrequency of blood sugar determination. S.B.B.

Brown, Arnold L., Jr. (Mayo Clinic, Rochester, Minn.): CONTRIBUTIONS OF ELECTRON MICROSCOPY TO LIPID PHYSIOLOGY AND ATHEROSCLEROSIS. Illinois M.J. *119*:378-79, June 1961.

The electron microscope has become a valuable tool in the study of lipid metabolism and atherosclerosis. Hypercholesterolemia has been shown experimentally to result in aggregation of lipids on the surface of the vascular endothelial cell. Subsequently the lipid is taken into the cell by pinocytosis. It has been suggested that the intestinal absorption of fat is abnormal in patients with atherosclerosis. Electron microscopy has shown the "brush border" of the intestinal cell to consist of numerous "microvilli." The latter absorb lipid by pinocytosis and the lipid then passes through the cell to the lacteals. Absorbed butter and corn oil show considerable differences in their appearance in the intestinal epithelial cells. More information is expected from continuing studies with electron microscopy. H.L.W.

Chesley, Leon C.; Kaufmann, Peter; and Pauerstein, Carl (Dept. of Obstetrics & Gynecology, State Univ. of New York Downstate Med. Center & Kings County Hosp., Brooklyn, N. Y.): PROGRESSIVE RESISTANCE TO INTRAVENOUS TOLBUTAMIDE IN PREGNANCY. Metabolism *10*:454-63, June 1961.

Blood sugar levels 20, 30, 40 and 60 minutes after intravenous injection of 1 gm. of tolbutamide were determined in forty-six nondiabetic pregnant women, ten diabetic pregnant women, and twenty-seven nonpregnant nondiabetic women. Resistance to the hypoglycemic action of tolbutamide appeared at about the midpoint in pregnancy, and in the last trimester nearly all subjects, diabetic and nondiabetic, gave "diabetic" responses to the test, as manifested by (1) significantly smaller decreases in blood sugar than those in nonpregnant subjects, (2) greater lags in hypoglycemic effect and (3) absence of a significant rebound within one hour. A lag in the hypoglycemic response was the first step in the development of tolbutamide resistance. Fourteen of the women tested in late pregnancy were retested twelve to thirty weeks after delivery. In every case the hypoglycemic response to tolbutamide was greater postpartum. Some recovery of sensitivity to tolbutamide appeared within six to twelve hours after delivery in the six patients tested during labor and shortly after delivery. Possible explanations of these findings are that placental insulinase may cause the tolbutamide resistance of late pregnancy or that pregnancy inhibits the pancreatropic effect of tolbutamide. C.A.R.

Cornblath, Marvin; Ganzon, Angelita F.; Nicolopoulos, Demetrios; Baens, Gloria S.; Hollander, Richard J.; Gordon, Mordecai H.; and Gordon, Harry H. (Dept. of Pediat. of Sinai Hosp. of Baltimore and Johns Hopkins Medical Institutions, Baltimore, Md.): STUDIES OF CARBOHYDRATE METABOLISM IN THE NEWBORN INFANT. III. SOME FACTORS INFLUENCING THE CAPILLARY BLOOD SUGAR AND THE RESPONSE TO GLUCAGON DURING THE FIRST HOURS OF LIFE. Pediatrics 27:378-89, March 1961.

The authors discuss the influence of such perinatal factors as pretreatment of the mother, mode of delivery, and age of the newborn on the infant blood sugar levels following delivery, and on the infant's responsiveness to high and lower doses of glucagon. Elevations of infant's blood sugar produced by glucose infusion to the mother persisted in the infant for only short periods of time and values were similar to infants of nontreated mothers by four to six hours after delivery. Infants given 30 μg./kg. of glucagon intravenously less than three hours after birth developed significantly lesser elevations of blood sugar than did those given glucagon six hours or more after birth. Labor was found to increase the infant's responsiveness to glucagon. Pretreatment of the mother with glucose did not influence the effect of glucagon on the infant. Large doses of glucagon (300 μg./kg.) abolished the differences in response associated with age or mode of delivery which were noted with smaller doses. T.G.S.

Dosekun, F. O. (Dept. of Physiol., University Coll., Ibadan, Nigeria): THE MEASUREMENT OF METABOLIC AND VASCULAR RESPONSES IN THE THYROID GLAND WITH OBSERVATIONS ON ITS RESPONSES TO INSULIN, GLUCOSE AND ADRENALINE. J. Physiol. *157*:504-12, August 1961.

The metabolic heat production and blood flow of the thyroid gland were measured in anesthetized dogs by internal calorimetry. An intravenous infusion of glucose increased both the heat production and blood flow of the thyroid gland. Insulin, when given in a dose of 1 to 3 units/kg. body weight intravenously, with or without an infusion of glucose, depressed the metabolic heat production of the thyroid gland, but had no consistent effect on blood flow. H.T.N.

Eichhorn, J.; and Hechter, O. (Worcester Foundation for Experimental Biology, Shrewsbury, Mass.): INSULIN-INDUCED ACCUMULATION OF D-XYLOSE AGAINST AN APPARENT CONCENTRATION GRADIENT IN DIAPHRAGM MUSCLE, IN VIVO. J. Gen. Physiol. *45*:15-22, September 1961.

D-xylose-1-C^{14} in a dose of 0.8 to 1.1 mg. per rat, was injected subcutaneously in intact, hypophysectomized, or adrenalectomized animals, after functional nephrectomy. Extracellular space was measured by subcutaneous injection of insulin-C^{14} into other rats. Samples of plasma, diaphragm and gastrocnemius were taken ninety to 100 minutes after tracer injection for analysis of radioactivity. In all types of animals studied, the gastrocnemius exhibited a small apparent intracellular distribution of D-xylose, and this was markedly increased by the intraperitoneal administration of insulin. The intracellular D-xylose of gastrocnemius did not exceed the concentration in plasma. In all animals the average D-xylose concentration in the cell water of diaphragm was higher than that in gastrocnemius; when insulin was administered, the D-xylose concentration in diaphragm cells increased up to two times the concentration found in plasma at the time of sampling. The apparent intracellular concentration of D-xylose in diaphragm was higher than the plasma level even when

nonlabeled D-xylose was given to increase the total plasma level to around 2.2 mg. per ml. H.T.N.

Finley, John K. (Hahnemann Med. Coll. and Hosp., Philadelphia, Pa.): THE DISORDER OF FAT TRANSPORT IN DIABETES MELLITUS, ITS SIGNIFICANCE AND CORRECTION. Angiology *12*:127-29, April 1961.

Brief general clinical review of the problem. The author also reports on sublingual heparin therapy for diabetic retinopathy in five patients. Observations on the control of diabetes, funduscopic examinations and chylomicron counts are mentioned. After one year of therapy, four of the five patients were improved. G.D.M.

Fisher, R. B.; and Williamson, J. R. (Dept. of Biochemistry, Univ. of Oxford, Oxford, England): THE EFFECTS OF INSULIN, ADRENALINE AND NUTRIENTS ON THE OXYGEN UPTAKE OF THE PERFUSED RAT HEART. J. Physiol. *158*:102-112, September 1961.

Isolated rat hearts were perfused with Krebs-Ringer bicarbonate solution at 38.5° C. In the absence of substrates in the perfusion medium the oxygen uptake of the heart fell after forty to ninety minutes of perfusion, but could be maintained for three hours if nutrients were added. The rate of endogenous respiration in the period between fifteen and sixty minutes of perfusion was not altered by the addition of glucose and insulin, or of succinate or β-hydroxybutyrate to the perfusion fluid. The oxygen uptake was slightly lowered in the presence of acetoacetate; 10^{-5} M2,4-dinitrophenol increased the oxygen uptake by about 40 per cent without affecting the flow rate and 10^{-6} M adrenaline greatly increased the flow rate and the oxygen uptake of the rat heart. It is concluded that insulin does not act by stimulating oxidative metabolism. H.T.N.

Fisher, R. B.; and Zachariah, P. (Dept. of Biochemistry, Univ. of Oxford, Oxford, England): THE MECHANISM OF THE UPTAKE OF SUGARS BY THE RAT HEART AND THE ACTION OF INSULIN ON THIS MECHANISM. J. Physiol. *158*:73-85, September 1961.

Measurements have been made of the time course of penetration of L-arabinose and D-xylose into the isolated perfused rat heart. The addition of insulin, 0.2 milliunits per ml., to the perfusing fluid increased the rate of accumulation of the pentoses intracellularly. The addition of glucose to the perfusate decreased the rate of penetration of the pentoses, and galactose exerted a similar but smaller effect. When the heart was equilibrated with galactose prior to addition of pentose to the perfusing fluid, the pentose accumulated in the muscle cells at a slightly faster rate than in controls without prior addition of galactose. These findings are consistent with a carrier hypothesis of membrane transport of sugars. H.T.N.

FitzGerald, M. G.; Malins, J. M.; and O'Sullivan, D. J. (General Hosp., Birmingham, England): PREVALENCE OF DIABETES IN WOMEN THIRTEEN YEARS AFTER BEARING A BIG BABY. Lancet *1*:1250-52, June 10, 1961.

Glucose-tolerance tests were carried out on sixty-one women thirteen years after they had given birth to a baby weighing over 10½ lb. Twenty of the women had definite or probable diabetes. Though abnormal glucose tolerance was rare under the age of forty-five, over half of those examined over forty-five were abnormal. Both the normal and diabetic women were obese, highly parous, and often had a relative known to have diabetes. Probably, therefore, many of the women who were then normal are destined to develop diabetes, but the separate influence of having a big baby could not be assessed in isolation from other factors known to dispose to diabetes. W.R.K.

Gais, Elmer S. (Diabetic Clinic, Montefiore Hosp. & Medical Serv. of Bellevue & Univ. Hosp., New York, N. Y.): NONGLUCOSE MELITURIAS. New York J. Med. *61*:2794-95, Aug. 15, 1961.

The author reviews the subject of nonglucose meliturias. A schema is presented for identifying reducing sugars in human urine and there is a discussion of the various benign meliturias including pentosuria, galactosuria, fructosuria, mannoheptulosuria, lactosuria, maltosuria, sucrosuria, and excretion of glucuronates in the urine. Pathologic physiology and genetic aspects of these disorders are briefly discussed. C.A.R.

Gryglewski, R.; and Duncan, L. J. P. (Depts. of Pharmacol. and Therapeutics, Univ. of Edinburgh, U. K.): THE EFFECT OF TOLBUTAMIDE AND INSULIN ON THE REDUCED GLUTATHIONE CONTENT OF THE BLOOD AND LIVER OF NORMAL AND ALLOXAN-DIABETIC RATS. Quart. J. Exper. Physiol. *46*:150-55, April 1961.

The blood reduced glutathione (GSH) concentration of normal rats was reduced by the intracardiac injection of tolbutamide but not by insulin or sulfadiazine. Tolbutamide did not lower the blood GSH of severely alloxan-diabetic rats. No significant change in the GSH content of the pancreas, kidney or liver was observed in normal rats following the injection of tolbutamide or sulfadiazine. The fall in the GSH content of liver homogenates incubated in vitro was accelerated by tolbutamide except when glucose was added to give a high concentration in the homogenate. Perfusion of the liver in situ with insulin or sulfadiazine did not alter its GSH content but perfusion with tolbutamide significantly reduced the GSH content. W.R.K.

Haimovici, Henry (Vascular Serv., Surg. Div., Montefiore Hosp., New York, N. Y.): PERIPHERAL ARTERIAL DISEASE IN DIABETES. New York J. Med. *61*:2988-99, Part I, 1961.

Current concepts of the basic pathology of the peripheral arterial disease in diabetes mellitus are reviewed. It is pointed out that in addition to the atherosclerotic lesions involving the medium and large-sized arteries, the smaller arteries (digital, cutaneous, and muscular) display lesions characterized by endothelial proliferation. Furthermore, the atherosclerotic changes in diabetic patients are more diffuse and involve more often the medium-sized arteries of the leg and foot. Its widespread distribution, its rapid rate of development, and the frequent involvement of the terminal arteriolar bed are the three major factors which may account for the severity of the peripheral arterial disease in diabetic patients.

In addition to arteriosclerosis obliterans the clinical manifestations may include also those of peripheral neuropathy and infection. It is emphasized that the clinical picture is usually a complex one in which the preceding three types of lesions are combined in varying degrees, although any one of them may be present independent of each other.

An outline of the basic principles of medical and surgical procedures is presented. Special emphasis is placed on conservative management with a view to salvaging the limb.

While much remains to be accomplished in basic research

concerning the relationship of diabetes mellitus to the development of atherosclerosis, nevertheless remarkable strides over the past decade have been achieved in the management of the peripheral arterial disease and its complications in diabetes. C.A.R.

Henderson, M. J.; Morgan, H. E.; and Park, C. R. (Dept. of Physiol., Vanderbilt University Sch. of Med., Nashville, Tenn.): REGULATION OF GLUCOSE UPTAKE IN MUSCLE. V. THE EFFECT OF GROWTH HORMONE ON GLUCOSE TRANSPORT IN THE ISOLATED, PERFUSED RAT HEART. J. Biol. Chem. 236:2157-61, August 1961.

Glucose uptake by the isolated heart from hypophysectomized rats was stimulated by addition of bovine growth hormone to the perfusion medium. No effect was found when the normal heart was employed. Stimulation also failed to occur in muscle from diabetic animals, and hypophysectomy did not improve uptake in diabetic animals. Insulin stimulated glucose uptake, but the effect of growth hormone did not require the presence of insulin in the tissues. The sensitivity of membrane transport to the presence of insulin was reduced by addition of either growth hormone or cortisone. The late decrease of glucose uptake in the rat diaphragm by growth hormone is thought to be unimportant. A.R.C., JR.

Henneman, Dorothy H.; and Shoemaker, William C. (Depts. of Anesthesia and Surgery, Peter Bent Brigham Hosp. and Harvard Med. Sch., Boston, Mass.; Dept. of Med., Seton Hall Coll. of Med., Jersey City, N. J.; Dept. of Exper. Surgery, Michael Reese Hosp. and Med. Center, Chicago, Ill.): EFFECT OF GLUCAGON AND EPINEPHRINE ON REGIONAL METABOLISM OF GLUCOSE, PYRUVATE, LACTATE, AND CITRATE IN NORMAL CONSCIOUS DOGS. Endocrinology 68:889-98, June 1961.

Arteriovenous concentration gradients and blood flow were measured simultaneously across liver and hindquarter of dogs. In the nonfasting state glucagon caused the liver to release glucose and take up pyruvate, lactate, and citrate. On the other hand, glucagon caused an increase in the lactate and citrate released by the hindquarter and a slight increase in pyruvate uptake. Hind limb blood flow decreased.

Fasting diminished the glucagon effect on regional glucose metabolism, and blood flow but not on regional pyruvate, lactate, or citrate metabolism. Thus the effects of glucagon on peripheral glucose uptake and blood flow are related to changes in the level of blood glucose while the effects on pyruvate, lactate and citrate are not.

Epinephrine decreased the uptake of pyruvate and glucose by the hindquarter and in small doses increased the net peripheral production of pyruvate and lactate while increasing their uptake by the liver. H.L.W.

Hines, James J.; and Kessler, Donald L. (Depts. of Med., St. Joseph Hosp. & Northwestern Univ. Med. Sch., Chicago, Ill.): UNEXPLAINED REMISSION OF LONG STANDING SEVERE DIABETES MELLITUS. Ann. Int. Med. 55:314-16, August 1961.

The authors describe the clinical course of a thirty-eight-year-old white female with diabetes of twenty-one years' duration who had a remission which to date has lasted eleven years. Oral and intravenous glucose tolerance test while on a free diet are both normal. No renal disease was present. S.B.B.

Kaplan, Norman M.; Parker, Gerald W.; and Beering, Steve C. (Endocrinology Serv., Dept. of Med., U.S. Air Force Hosp., U.S.A.F. Aerospace Med. Center, Lackland Air Force Base, Tex.): HYPOPITUITARISM AND DIABETES MELLITUS: A CASE REPORT WITH OBSERVATIONS ON METABOLIC INTERRELATIONSHIPS. Metabolism 10:447-53, June 1961.

During the twenty-eighth week of the second pregnancy of a twenty-four-year-old diabetic, who required 45 units of insulin daily before pregnancy, a rapid decrease in insulin requirement (from 75 to 40 units) occurred. After delivery, hypoglycemic episodes were frequent with 15 units insulin daily, and the signs and symptoms of panhypopituitarism developed. Urinary gonadotropins, BMR, I^{131} uptake before and after TSH, urinary hydroxycorticoids and 17-ketosteroids before and after ACTH, endometrial biopsy, insulin tolerance, and the SU-4885 test for pituitary ACTH reserve were consistent with panhypopituitarism; however, epinephrine produced a sharp rise in blood nonesterified fatty acids. Neither thyroid nor gonadal hormone replacement therapy affected the insulin requirement; but physiologic doses of hydrocortisone increased it. On a daily regimen of 25 mg. hydrocortisone, 180 mg. desiccated thyroid, cyclic estrogen and progesterone, and 2.5 mg. fluoxymesterone q. three days, 25 units of insulin were required; and all evidences of thyroid and gonadal insufficiency disappeared. The failure of hormonal replacement therapy to restore the patient's insulin requirement to that existing before the onset of hypopituitarism is thought to reflect the deficiency of growth hormone. The combination of vascular occlusive disease, disturbed glucose metabolism in the pituitary, and pituitary hypertrophy presumably dispose the pregnant diabetic to pituitary necrosis. C.A.R.

Landau, Bernard R.; Leonards, Jack R.; Craig, James W.; Shepardson, Charles R.; Moriwaki, Takeshi; and Miller, Max (Dept. of Med. & Biochemistry, Western Reserve Univ., Cleveland, Ohio): DIET AS A FACTOR IN THE RESPONSE OF DOG AND MAN TO TOLBUTAMIDE ADMINISTRATION. Metabolism 10:464-68, June 1961.

In eight dogs and nine normal human subjects, the blood glucose responses to intravenous tolbutamide were compared after both high and low carbohydrate diets, given alternately for five-day periods. The response to tolbutamide, as evidenced by both a slower decline and failure to attain as low a blood glucose concentration, was less for a given dog or subject after the low carbohydrate diet than after the high carbohydrate diet. It is concluded that the dietary state of individuals is important in evaluating the response to tolbutamide. If the possible effect of diet is not considered, mild diabetes may be mistakenly diagnosed in normal subjects. These effects of diet on tolbutamide response are consonant with the concept of insulin release as a mechanism of tolbutamide action, since insulin sensitivity is known to be related to carbohydrate intake. C.A.R.

Laron, Z. (Pediat. Metabolic and Endocrine Clinic, Petah Tikva, Israel): ESSENTIAL BENIGN FRUCTOSURIA. Arch. Dis. Childhood 36:273-77, June 1961.

Essential fructosuria is a benign error in metabolism characterized by an inability to utilize fructose completely because of a deficiency of the hepatic enzyme fructokinase. The author studied interrelationships between glucose and fructose in an eleven-year-old girl with fructosuria by means of fructose loading, glucagon injection, insulin infusion, and tolbutamide administration. When hyperglucosemia was induced after simultaneous glucose and fructose loading, fructosuria was greater than when fructose was given alone. Glucagon induced

hyperglucosemia did not result in increased fructosuria but blood glucose did not rise as high. When tolbutamide or insulin were given to produce low blood glucose levels fructose loading resulted in quantitatively less fructosuria. The decreased fructose tolerance caused by glucose administration is not readily explained but could be caused by an inhibitory action of glucose on the phosphorylation of fructose by hexokinase. Conversely, the increased fructose tolerance induced by hypoglucosemia could be due to greater tissue availability of hexokinase. T.G.S.

Lowbeer, Leo (Hillcrest Med. Center, Tulsa, Okla.): HYPOGLYCEMIA-PRODUCING EXTRAPANCREATIC NEOPLASMS: A REVIEW. Am. J. Clin. Path. *35*:233-43, March 1961.

The author reviews forty-nine large extrapancreatic neoplasms, including twenty-six of mesodermal origin. Explanations for hypoglycemia are discussed. G.D.M.

Ludwig, George D.; and Elsom, Katharine O. (Univ. of Pennsylvania, Philadelphia, Pa.): MEDICAL CORRELATION CLINICS: DIABETES AND ITS COMPLICATIONS. Am. Pract. & Digest Treat. *12*:469-80, July 1961.

This is a round table discussion of the structural complications of diabetes mellitus. With respect to pathogenesis of the small vessel complications, it was stressed that normoglycemia should not necessarily be equated with normalization of lipid and mucopolysaccharide metabolism. This was based on evidence that collateral metabolic pathways which are not insulin dependent, may influence both these biochemical areas in various directions. The implications of this line of thinking upon therapeutic methods and aims were briefly discussed. S.B.B.

MacDowell, Muriel; and Oliver, Jean (Renal Res. Unit, Res. Dept., CIBA Pharmaceutical Products, Inc., Summit, N. J.): THE STRUCTURAL AND FUNCTIONAL ASPECTS OF THE HANDLING OF GLUCOSE BY THE NEPHRONS AND THE KIDNEY AND THEIR CORRELATION BY MEANS OF STRUCTURAL-FUNCTIONAL EQUIVALENTS. J. Clin. Invest. *40*:1093-112, July 1961.

Based on the familiar observations that glucose is handled in the kidneys by two structures, the glomeruli and the proximal convoluted tubules, an attempt was made to investigate the relationship between structural size of these units and physiological activity in glucose excretion and absorption. The latter were derived from old animal kidney and nephron studies in vivo of several parameters of kidney function. Detailed microdissection and measurement of three human kidneys were made with respect to length and volume of proximal convoluted tubules and surface and volume of the glomerular components. Of these the first proved to be the most constant feature. A distinct correlation was found between functional activity and anatomical size. The larger the proximal convoluted tubules the lower the ratio of relative glomerular activity was found. S.B.B.

Morgan, H. E.; Regen, D. M.; Henderson, M. J.; Sawyer, T. K.; and Park, C. R. (Dept. of Physiol., Vanderbilt University Sch. of Med., Nashville, Tenn.): REGULATION OF GLUCOSE UPTAKE IN MUSCLE. VI. EFFECTS OF HYPOPHYSECTOMY, ADRENALECTOMY, GROWTH HORMONE, HYDROCORTISONE, AND INSULIN ON GLUCOSE TRANSPORT AND PHOSPHORYLATION IN THE PERFUSED RAT HEART. J. Biol. Chem. *236*:2162-68, August 1961.

Further investigations were undertaken on the influence of adrenal cortical and pituitary secretions upon uptake of glucose by the isolated, perfused rat heart. Alloxan-diabetic animals exhibited a 70 per cent reduction in glucose uptake. Neither hypophysectomy nor adrenalectomy affected this finding, indicating that the inhibition is due to insulin deficiency. Marked stimulation of glucose uptake was noted in both adrenalectomized and hypophysectomized rats following the addition of insulin. This was accompanied by a rise in intracellular glucose, compatible with the acceleration of transport. Decrease in phosphorylation in the diabetic animals was restored to normal by either insulin, hypophysectomy or adrenalectomy. Phosphorylation was further depressed in the hypophysectomized-diabetic rats by administration of either growth hormone or hydrocortisone. If sufficient insulin was added to the medium, the effect of these hormones failed to occur. Glucagon in vitro increases phosphorylation in the diabetic heart. In the normal animal, tissue glucose uptake is limited because insulin secretion antagonizes the effect of pituitary and adrenal hormones. In the diabetic animal, the tissues fail to take up glucose properly because of excessive adrenal cortical and pituitary activity, insulin deficiency and insensitivity of the tissues to insulin. A.R.C., JR.

O'Sullivan, John B. (Prenatal Metabolism Clin., Boston City Hosp., & Boston Univ. Sch. of Med., Boston, Mass.): GESTATIONAL DIABETES: UNSUSPECTED, ASYMPTOMATIC DIABETES IN PREGNANCY. New Eng. J. Med. *264*:1082-85, May 25, 1961.

Gestational diabetes is defined by the response to the glucose tolerance test. Of 20,070 pregnancies screened, it was estimated that gestational diabetes occurred in about one out of 116 pregnancies. Follow-up study of these patients showed an observed incidence of overt diabetes of 7.1 per cent in the postpartum period and 28.5 per cent after five and one-half years. Statistical correction with the use of the life table method revealed accumulative incidence of diabetes at five and one-half years to be 67 per cent. S.S.

Robinson, Geoffrey C.; and McConnell, Doreen (Dept. of Pediat., Faculty of Med., Univ. of B. C., Vancouver, B. C.): SIMULTANEOUS ONSET OF DIABETES MELLITUS AND THE NEPHROTIC SYNDROME. Canad. M.A.J. *85*:80-81, July 8, 1961.

Report of an eight-year-old girl with simultaneous onset of diabetes mellitus and nephrosis. The laboratory findings, response to therapy (steroid), and clinical course suggested the latter diagnosis rather than diabetic nephropathy. S.B.B.

Segal, Stanton; Berman, Mones; and Blair, Alberta (Clinical Endocrinology Branch & The Office of Mathematical Res., National Inst. of Arthritis & Metabolic Diseases, Bethesda, Md.): THE METABOLISM OF VARIOUSLY C^{14}-LABELED GLUCOSE IN MAN AND AN ESTIMATION OF THE EXTENT OF GLUCOSE METABOLISM BY THE HEXOSE MONOPHOSPHATE PATHWAY. J. Clin. Invest. *40*:1263-79, July 1961.

This is the first attempt to estimate the extent of glucose metabolized in the fasting human subject by the hexosemonophospate shunt using a kinetic analysis. Variously labeled C^{14} glucose was injected and $C^{14}O_2$ production, C^{14} disappearance and randomization of label in blood glucose, and the incorporation of radioactivity in serum lipids were measured. It was calculated that about 8 per cent of glucose is metabolized by this pathway in man. S.B.B.

Sokol, E. Marvin (Veterans Administration Hosp., Minneapolis, Minn.): DIABETES MELLITUS AND THE ADRENAL GLAND. Journal-Lancet *81*:241-48, June 1961.

There is a considerable influence of adrenal secretions on carbohydrate metabolism. Epinephrine causes glycogenolysis through activation of phosphorylase and interferes with the phosphorylation of free glucose by the presence of excess glucose-6-phosphate. Pheochromocytomas are accompanied by diabetes in about 10 per cent of cases.

Adrenal glucocorticoids increase the total synthesis of carbohydrate, increase protein catabolism, and in the diabetic animal increase ketone production. Cushing's syndrome is accompanied by carbohydrate abnormality in over 75 per cent of patients. Diabetes associated with Addison's disease is rare and is manifest by unusual insulin sensitivity, instability of blood sugar level and severe episodes of hypoglycemia.

Aldosterone is weak in its influence on carbohydrate metabolism but 30 per cent of patients with primary aldosteronism show decreased tolerance for carbohydrate. A case of aldosteronism with latent diabetes is reported.

Adrenocortical hormones may play a role in the complications of diabetes, but adrenalectomy and hypophysectomy have not been notably successful in relieving these complications. H.L.W.

Sprecace, George A.; Pennoyer, Douglass C.; and Thompson, James E. (Roosevelt Hosp., New York, N. Y.): FUNCTIONING ISLET-CELL CARCINOMA OF THE PANCREAS: PARTIAL LITERATURE REVIEW. Postgrad. Med. *30*:36-46, July 1961.

A patient with islet-cell carcinoma of the pancreas is described.

The presenting complaint was abdominal pain, and an abdominal tumor was palpated. Repeated surgical treatment was necessary for local recurrence, over a period of sixteen months. Hypoglycemia occurred only terminally at a time that massive metastatic invasion of the liver had occurred. Assay of the blood insulin (Dr. Renold) by the fat pad method revealed three times the normal average amount. S.B.B.

Sunderman, F. William, Jr.; and Sunderman, F. William (Jefferson Med. Coll., Philadelphia, Pa.): MEASUREMENT OF GLUCOSE IN BLOOD, SERUM, AND PLASMA BY MEANS OF A GLUCOSE OXIDASE-CATALASE ENZYME SYSTEM. Am. J. Clin. Path. *36*:75-91, July 1961.

The new procedure depends on oxidation of glucose to gluconic acid and hydrogen peroxide by action of glucose oxidase. The hydrogen peroxide induces oxidation of methanol to formaldehyde by action of catalase, present as a contaminant in preparations of glucose oxidase. Formaldehyde, produced by the coupled enzyme system, is measured by the chromotropic acid color reaction.

The authors document greater precision, sensitivity and specificity for their test than they found with two copper reduction technics and an automated ferricyanide reduction technic. Fluoride interference, noted with the glucose oxidase-peroxidase system, was not encountered by the new method. Of thirty-six compounds tested for interference with glucose chromogenticity, only ascorbic acid caused artifactitious elevation of blood glucose. G.D.M.

Traisman, Howard S.; and Newcomb, Alvah L. (Chicago, Ill.): THE EFFECT OF SAFFLOWER OIL UPON SERUM LIPIDS AND PROTEINS IN JUVENILE DIABETES MELLITUS. Northwestern Med. Sch. Quarterly Bull. *35*:143, Summer 1961.

Twenty patients with juvenile diabetes mellitus were studied. Half of these patients substituted an emulsion of safflower oil (SAFF 15 ml. three times daily) for an equivalent amount of fat in their diet while the other ten served as controls.

Initially mean values for blood lipids in all patients were within normal ranges for nondiabetic individuals. After twelve weeks the treated group showed a statistically significant fall in serum cholesterol, cholesterol esters, phospholipids, triglycerides, total lipid and the beta lipoproteins.

The substitution of unsaturated for saturated fat in the diet may be of value in treating hyperlipemia and possibly in the prevention of early atherosclerosis in juvenile diabetic patients. H.L.W.

Unger, Roger H.; Eisentraut, Anna M.; McCall, M. S.; and Madison, Leonard L. (Dept. of Med. & Radioisotope Lab., Veterans Hosp.; & the Dept. of Med., Univ. of Texas Southwestern Med. Sch., Dallas, Tex.): GLUCAGON ANTIBODIES AND AN IMMUNOASSAY FOR GLUCAGON. J. Clin. Invest. *40*:1280-89, July 1961.

An immunoassay of glucagon of great specificity and sensitivity is described. A method similar to that for insulin (Berson et al.) was possible because rabbits produced antibodies to glucagon (beef-pork). In virtually every category glucagon antibodies resembled insulin antibodies, except that they migrated in the gamma-globulin fraction. The method was confirmed by comparative assays by the respective methods of injected labeled and unlabeled glucagon. The method could detect as little as 50$\mu\mu$g. per ml. of beef-pork glucagon. There is evidently cross-reactivity of glucagon from different species. This enabled the investigators to attempt an assay of canine and human pancreases for glucagon. While the absolute figures may still be subject to revision, the assay showed a total pancreatic glucagon content of 22-36 μg., with the major portion present in the tail. S.B.B.

Vaughan, Martha (Sect. on Metabolism, Lab. of Cellular Physiol. and Metabolism, National Heart Inst., National Institutes of Health, Bethesda, Md.): EFFECT OF HORMONES ON GLUCOSE METABOLISM IN ADIPOSE TISSUE. J. Biol. Chem. *236*:2196-99, August 1961.

Uptake of glucose by epididymal fat bodies from male rats was stimulated by addition of epinephrin, norepinephrin, ACTH, glucose or serotonin. Presence of glucose inhibited free fatty acid release from adipose tissue, and this effect was increased by insulin. Glucagon increased the incorporation of C^{14} from labeled glucose into glycerol and fatty acid. Epinephrin decreased incorporation into fatty acid from glyceride. A.R.C., JR.

Wolter, J. Reimer (Dept. of Ophthalmic Surgery, Univ. of Mich. Med. Center, Ann Arbor, Mich.): DIABETIC CAPILLARY MICROANEURYSMS OF THE RETINA. Arch. Ophthalmol. *65*: 847-54, June 1961.

Retinas from two diabetic patients were digested in trypsin, leaving only the cobweb-like vascular network. Staining with silver carbonate revealed intervascular mesodermal strands, more coarse than those seen in the normal nondiabetic retina. Microscopic examination and photomicrographs support the theory of Ashton that these strands may contribute to formation of microaneurysms by producing bulging of the capillary wall. A.R.C., JR.

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10th POSTGRADUATE COURSE

As previously announced, the Tenth Postgraduate Course of the American Diabetes Association, entitled "Diabetes in Review: Clinical Conference, 1962," will be held in Detroit and Ann Arbor, Michigan, Jan. 17, 18, and 19, 1962. In addition to the Scientific Program, which was published together with other pertinent information in the September-October issue of the Journal, a number of special events have been scheduled.

WOMEN'S ACTIVITIES

Mrs. Laurence F. Segar, a member of the Local Committtee, is in charge of activities for women.

On Wednesday, January 17, there will be a tour of Greenfield Village, at a price of $6.75, which will include village and museum admission, carriage ride through the village, and lunch in the Country Kitchen at the Clinton Inn on the Village Green. Private cars will leave downtown Detroit at 10:30 a.m.

On Thursday, January 18, private cars will leave downtown Detroit for Ann Arbor at 11 a.m. There will be a visit to the Phoenix Memorial Laboratories and the Ford Nuclear Reactor, at a charge of $6.00, which will include lunch at 1 p.m. in the Kalamazoo Room, Women's League. A bus tour of the University of Michigan campus from 2 to 4 p.m. will be followed by tea at the home of Dr. and Mrs. Stefan S. Fajans.

On Friday, January 19, guests will be taken to points of interest and shopping in Detroit by private car at no charge.

LOCAL COMMITTEE

Frank S. Perkin, M.D., is Chairman of the Local Committee, whose members include Daniel E. Cohn, M.D., Stefan S. Fajans, M.D., Miss Mary M. Harrington, Henry D. Kaine, M.D., William M. LeFevre, M.D., Laurence F. Segar, M.D., Mrs. Laurence F. Segar, Herschel A. Shulman, M.D., Nelson M. Taylor, M.D., George C. Thosteson, M.D., and Fred W. Whitehouse, M.D.

BANQUET SPEAKER

Bob Considine, well-known columnist and journalist, will be the Banquet Speaker on Wednesday evening, January 17.

PRINTED PROGRAM

The program which includes an application form has been mailed to all members of the Association and to subscribers of DIABETES. This form should be filled out and mailed together with the fee ($40 for members of the American Diabetes Association and $75 for nonmembers) to the American Diabetes Association, 1 East 45th Street, New York 17, N.Y. Applications will be accepted in the order received and registrations officially confirmed. A hotel reservation card will be sent to registrants.

22nd ANNUAL MEETING

Chicago will be the site of the Twenty-second Annual Meeting of the American Diabetes Association, June 23-24, 1962, just prior to the annual session of the American Medical Association. The Conrad Hilton hotel will serve as headquarters for the meeting. Scientific Sessions will be held on Saturday morning, June 23, and Sunday morning and afternoon, June 24. The Annual Banquet and Social Hour, to which Members, their wives (or husbands) and friends are cordially invited, will be given Saturday evening, June 23. A list of papers to be presented at the Scientific Sessions, as well as

other information, will be published in a future issue of DIABETES. The Program itself, which includes abstracts of all papers, will be sent to Members prior to the Meeting.

NEW PROCEDURE FOR SUBMISSION OF ABSTRACTS

Because of the ever-increasing number of abstracts of papers in the field of diabetes and metabolism that are submitted for publication in the Program of the Annual Meeting of the American Diabetes Association, and because of the consequent urgent need for some reasonable methods of limiting their number and maintaining their quality, the ADA Council at the 21st Annual Meeting in June approved a new procedure for the submission of abstracts as recommended by the Committee on Scientific Programs, chaired by Dr. Jerome W. Conn.

These procedures, which are given below in full, have also been sent to the membership in a communication from Dr. Thomas P. Sharkey, the present Chairman.

1. An active member may submit no more than two abstracts, one as author and one as co-author. He may, in lieu of submitting abstracts, introduce no more than two abstracts on behalf of nonmembers. When submitting one abstract he may introduce one abstract by a nonmember.

2. While utilizing his prerogative with respect to a total of two abstracts, the name of a member may appear once again as a co-author, providing that the abstract was submitted by another member. He may not, however, have another abstract of his own introduced by another member.

3. Abstracts of nonmembers must be introduced by active members in accordance with #1 (see above)-and are limited to one for a nonmember.

4. Abstracts are limited to 300 words. Those exceeding 300 words will not be accepted. The number of words contained in the abstract (excluding the title and authors) should appear at the end of the abstract.

5. Abstracts must be double-spaced and seven copies must be submitted.

6. The heading of abstracts should be in the following form:
   a. Title(s), first letters of major words being capitalized.
   b. Author(s) name(s), no degrees (e.g., M.D.), or academic titles should be included.
   c. Active members serving as authors or co-authors should be indicated by an asterisk.
   d. City and State of origin of work.

7. Abstracts accepted will be printed as submitted to the Chairman of the Committee on Scientific Programs. They should be carefully written and edited prior to submission. Changes may not be made after they have been submitted.

8. Abstracts must be postmarked not later than March 1, 1962.

9. Abstracts should not contain acknowledgments, diagrams, charts, tables, or references. Charts or tables may be sent to aid the Committee in selecting abstracts for the program, but reference to charts, tables, etc., should not be made in the text since they will not appear in the published abstracts.

10. Papers are to be presented by the first author listed. Presentation at the Annual Meeting will be strictly limited to fifteen minutes to allow time for discussion.

Abstracts should be sent as soon as possible, but not later than March 1, 1962, to Dr. Sharkey in care of the national office at 1 East 45th Street, New York 17, N.Y.

1961-62 GRADUATE AND MEDICAL STUDENT, INTERN AND RESIDENT ESSAY CONTESTS

Medical students, interns, residents throughout the period of their residency, physicians within two years after their graduation from medical school, and graduate students in the basic sciences are invited to enter the tenth Graduate and Medical Student, Intern and Resident Essay Contests.

The following prizes are being offered:

- $250 Prize—for the best paper reporting original work, whether laboratory investigation or clinical observation.
- $100 Prize—for the best review article or case report.

Prize winners, as well as those receiving honorable mention, will also be given a one-year subscription to DIABETES: *The Journal of the American Diabetes Association.*

The papers will be judged on the basis of value of the material and manner of presentation. Any subject relating to diabetes and basic metabolic problems may be selected.

Manuscripts must not have been previously published. Appropriate papers will be considered for publication in DIABETES.

Entrants should submit the original and two copies of a typewritten, double-spaced manuscript with a letter of transmittal by March 15, 1962, to: Committee on Scientific Awards, American Diabetes Association, 1 East 45th Street, New York 17, N.Y.

BOARD OF STATE GOVERNORS

New officers were elected to the Board of State Governors on June 22, 1961, at the time of the Twenty-first Annual Meeting. W. Bernard Yegge, M.D., Denver, Colorado, was elected Chairman.

Addison B. Scoville, Jr., M.D., Nashville, Tennessee, was elected Vice Chairman, and George W. Welsh, III, M.D., Burlington, Vermont, was elected Secretary of the Board.

Governors who will serve for the organizational year 1961-62 are:

Term Expiring June 1963

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Alabama	Samuel Eichold
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Arkansas	Hal Dildy
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Northern & Nevada	Mervyn U. Schwartz
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Illinois	
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Southern	Thomas D. Masters
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Term Expiring June 1964	
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Kentucky	Carlisle Morse
Louisiana	Sol B. Stern, Jr.
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Nevada	See Northern California
New Hampshire	Jackson W. Wright
New Jersey	Everett O. Bauman
New York	
Eastern	Milton B. Handelsman
Western	Joseph L. Izzo
North Carolina	William M. Nicholson
North Dakota	Donald M. Barnard

Term Expiring June 1962	
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Oklahoma	Robert C. Lawson
Oregon	Wm. Richey Miller
Pennsylvania	
Eastern	Frederick G. Helwig
Western	L. Lewis Pennock
Rhode Island	Louis I. Kramer
South Carolina	Robert Wilson
South Dakota	Everett W. Sanderson
Tennessee	Addison B. Scoville, Jr.
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Utah	William H. Bennion
Vermont	George W. Welsh, III
Virginia	William R. Jordan
Washington	Howard M. Hackedorn
West Virginia	Richard N. O'Dell
Wisconsin	Bruno J. Peters

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DECEMBER 1 DEADLINE FOR 1962 LILLY AWARD NOMINATIONS

Nominations for the sixth Lilly Award which will be given at the Twenty-second Annual Meeting of the American Diabetes Association, June 23-24, 1962, in Chicago, must be received on or before Dec. 1, 1961. This annual award is supported by Eli Lilly and Company and consists of $1,000 and a medal. The following stipulations govern the contest:

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

NEW MEMBERSHIP DIRECTORY

An up-to-date Membership Directory of the American Diabetes Association, including the names of all members of record as of March 1, 1961, will be sent to all members without charge in December. The Directory will include an alphabetical and geographical listing of Active Members, an alphabetical listing of Associate and Corporate Members, and other information of organizational interest.

DIABETES INDEX

Many subscribers to DIABETES: *The Journal of the American Diabetes Association* customarily bind their issues for the year after they receive the Subject and Author Index for that volume. It is expected that the Index for Volume 10, 1961, will be completed and mailed approximately March 1. No charge is made for the Index to members and to direct subscribers of the Journal.

NEW MEMBERS

The following were elected as of Oct. 1, and Nov. 1, 1961:

Active

California	
Brown, Royal L.	San Bernardino
Hyatt, Herman W.	Bakersfield
Colorado	
Schemmel, Janet E.	Denver
Illinois	
Sobel, Gerald W.	Chicago
Iowa	
Graham, Judith	Iowa City
Kansas	
Sumner, Marion M.	Hutchinson
Massachusetts	
Younger, Mary D.	Boston
Minnesota	
Morgan, Carl R.	Minneapolis
Missouri	
Blumenthal, H. T.	St. Louis
Field, Morton H.	Richards-Gebaur AFB
New Mexico	
Whitcomb, John G.	Albuquerque
New York	
Beck, John E.	New York
Georgeson, L. W.	Riverhead
O'Connor, Charles F.	Eggertsville
Shalsha, Kurt G.	New York
Streeten, D. H. P.	Syracuse
Williams, R. P.	New York
Ohio	
Visintine, R. E.	Columbus
Oregon	
Garland, James	Hillsboro

Pennsylvania

Cherner, Rachmel	Philadelphia
Gilgore, Sheldon G.	Philadelphia

Wisconsin

Gedge, Stafford W.	Marshfield

Other Countries

Brazil

Coronho, Victor	Belo Horizonte
Purisch, Moyses	Belo Horizonte

NEWS OF AFFILIATE ASSOCIATIONS

The ALABAMA DIABETES ASSOCIATION presented its sixth "Seminar on Diabetes Mellitus" Sept. 17, 1961, at the Liberty National Auditorium in Birmingham. Leon S. Smelo, M.D., Birmingham, President of the Association, presided and the following program was given: "Insulin Antagonists," by Rosalyn Yalow, Ph.D., New York; "Diabetes Mellitus—What Is It?" by Holbrooke Seltzer, M.D., Dallas; "Current Status of Oral Hypoglycemic Agents," by Samuel Beaser, M.D., Boston; "Questions and Answers," by Drs. Yalow, Seltzer and Beaser. Buris R. Boshell, M.D., Birmingham, was program chairman.

The FLORIDA DIABETES ASSOCIATION held its ninth annual "Diabetes Seminar" Oct. 19-20, 1961, at the Balmoral Hotel in Miami Beach. The October 19 morning program, with Morris B. Seltzer, M.D., Daytona Beach, as moderator, included "Critical Assessment of the Role of Oral Hypoglycemic Agents in Management of Diabetes Mellitus," by Max Miller, M.D., Cleveland; "Use of Tolbutamide in the Treatment of Young Patients with Mild Asymptomatic Diabetes Mellitus," by Stefan S. Fajans, M.D., Ann Arbor; "Endocrine Factors Affecting Free Fatty Acid Concentrations in Blood," by Richmond W. Smith, Jr., M.D., Detroit.

George H. Garmany, M.D., Tallahassee, was moderator of the afternoon program when the following papers were read: "Metabolism of Fructose in Normal and Diabetic Subjects," and "The Pregnant Diabetic—Considerations on Management," by Dr. Miller; "Sequellae of Hypoglycemia" by Joseph C. Shipp, M.D., Gainesville.

Sidney Davidson, M.D., Lake Worth, was moderator of the morning session on October 20, which included "Albright's Hereditary Osteodystrophy," by Joel B. Mann, M.D., and A. Gorman Hills, M.D., both of Miami; "Investigative Studies of Patients with Renal Calculi," by William C. Thomas, Jr., M.D., Gainesville; "The Natural History of Senile Osteoporosis," by Dr. Smith; "Hypothyroidism, A Not Uncommon Cause of 'Resistant Anemia,'" by Fred Mathers, M.D., Orlando. The afternoon program, with T. F. Hahn, Jr., M.D., DeLand, as moderator, followed the annual meeting of the Association. Dr. Fajans spoke on "Diagnostic Value of Sodium Tolbutamide in Hypoglycemic States," and "Production and Mechanism of Experimentally Induced Leucine Hypoglycemia"; and John F. Munroe, M.D., Gainesville, on "Significance of Hyperlipemia and Response to Treatment in the Diabetic Patient."

The NEW ENGLAND DIABETES ASSOCIATION held its Springfield Clinical Meeting at the Springfield Municipal Hospital, Nov. 14, 1961, at 8:30 p.m. Frank Hurley, M.D., President of the Springfield Academy of Medicine, was chairman of the panel discussion on diabetes. Panelists were Simeon Locke, M.D., Boston: "Diabetic Neuropathy"; Alexander Marble, M.D., Boston: "Present Status of Oral Hypoglycemic Agents"; and Searle B. Rees, M.D., Boston: "Recent Studies Regarding Diabetic Angiopathy." A clinic was held at 5 p.m. to discuss a case related to one of the subjects of the evening program.

The Association held its fall clinical meeting at the Jimmy Fund Building, Children's Medical Center, Boston, at 8:15 p.m. Oct. 31, 1961. Dr. Marble was chairman of the panel discussion on neurological problems in diabetes mellitus. Panelists included Ole J. Rafelsen, M.D., Aarhus, Denmark: "For and Against the Direct Effect of Insulin on Central Nervous System"; Richard A. Field, M.D., Boston: "Insulin Response of Isolated Sciatic Nerve"; and Donald G. Lawrence, M.D., Boston: "Clinical and Physiological Studies of Neuropathy in Diabetes Mellitus." Dr. Locke was the discussor.

NEWS NOTES

NIH PERSONNEL CHANGES

Ralph Knutti, M.D., since 1960 associate director of the National Institute of Arthritis and Metabolic Diseases, has been appointed Director of the National Heart Institute.

John F. Sherman, Ph.D., since 1958 deputy chief of Extramural Programs for the National Institute of Arthritis and Metabolic Diseases, has been appointed associate director for Extramural Research for the National Institute of Neurological Diseases and Blindness.

PERSONALS

EDWARD L. BORTZ, M.D., Philadelphia, will speak on "Creative Aging Through Research," at the Annual Convention of the National Society for Crippled Children and Adults, which will be held at the Denver Hilton hotel, Denver, Nov. 17-21, 1961.

NECROLOGY

CHARLES R. ARP, Atlanta, Georgia, born February 12, 1913.

NORMAN JOLLIFFE, Director of the Bureau of Nutrition, Department of Health of The City of New York, died Aug. 1, 1961, in New York City. The Department of Health invites the friends and associates of Dr. Jolliffe to join in paying tribute to his memory on Friday, Dec. 15, 1961, at 8:30 p.m. at Hosack Hall, New York Academy of Medicine, 103rd Street and Fifth Avenue, New York.

JULIO F. SCHUTTE, Miami Beach, Florida, born August 12, 1905.

WILLIAM M. SHEPPE, Wheeling, West Virginia, born July 2, 1895.